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OBSERVATIONS ON *THEILERIA PARVA*, THE PARASITE OF EAST COAST FEVER OF CATTLE.

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(Two charts and four diagrams.)

ALTHOUGH a good deal has been written about East Coast Fever in cattle, the literature relating thereto contains very little direct information regarding the parasite which stands in causal relation to the disease. Robert Koch (1898), who was the first to observe the parasite in cases of East Coast Fever occurring in German East Africa, regarded it as but a variety of *Piroplasma bovis* (= *bigeminum*) and described the disease as Texas Fever. Theiler (1904) was the first to distinguish clearly East Coast Fever from Redwater. He stated that "the disease has nothing to do with Texas Fever or Redwater; it is a new disease due to a parasite different to the one found in Texas Fever." Koch (1903—1904), who gave the disease its distinctive name, reached the same conclusions as Theiler. The investigations of Theiler (1904) established the following facts: Cattle which are immune to Redwater are susceptible to East Coast Fever. East Coast Fever is not communicable by blood inoculations (30 experiments, wherein 5 to 2000 c.c. of East Coast Fever blood were inoculated). He noted the absence of haemoglobinuria in the majority of animals affected with East Coast Fever, its presence in the majority of the animals affected with Redwater. He found that in most cases of East Coast Fever, there was no appreciable decrease in the number of red blood corpuscles, this being in marked contrast to what is observed in Redwater. Theiler noted that cattle might harbour both the parasites of Redwater (*P. bovis*) and those of East Coast Fever (bacillary forms = *T. parva*). The former generally appeared in the blood "only towards the end of the fever reaction in East Coast Fever," being previously latent in the animals which had been "salted" against Redwater. He distinguished "two groups of piroplasmosis," the *inoculable* (Redwater, canine and equine piroplasmosis) and the *uninoculable* (East Coast Fever) by injection of infected blood. The parasites in the latter are much smaller than in the former. He named the parasites of East Coast Fever *Piroplasma parvum.* Theiler distinguished the parasite of East Coast Fever from *P. bovis* because of the frequent occurrence of bacillary forms and the minute size of the parasite, but he nevertheless retained the new parasite in the genus *Piroplasma.*

The fact that East Coast Fever is not communicable by inoculations with large quantities of blood containing great numbers of the parasites led to other modes of inoculation being attempted. Thus, Koch and his collaborators inoculated splenic pulp and material obtained from lymphatic glands both subcutaneously and intravenously, but they obtained negative results. Theiler and Stockman (personal communication) and Nuttall (spleen pulp inoculated subcutaneously, unpublished) have also obtained negative results from similar inoculations. Meyer, of Pretoria (September, 1909), has, on the other hand, recently succeeded twice in transmitting East Coast Fever to cattle by intraperitoneal inoculations made by transplanting the whole spleen or portions of spleen removed from animals killed whilst dying from the disease.

We shall take occasion, in a future paper, to discuss the systematic position of the Haemocytozoa which are usually ranged under the genus *Piroplasma.* Nuttall and Graham-Smith (1906, p. 645) have laid stress upon the differences existing between *P. bovis* and the parasites of East Coast Fever both as to morphology and pathogenicity, and Bettencourt, França and Borges (1907) founded a new genus *Theileria* to include similar parasites to those of East Coast Fever. One of us (Nuttall, IX. 1908, p. 516) has expressed himself in complete agreement with the Portuguese authors in the matter of naming the parasite of East Coast Fever *Theileria parva.*

From a study of three typical species of *Piroplasma* (*P. canis, P. bovis,* and *P. pitheci*), Nuttall and Graham-Smith (1908, p. 141) concluded that the members of the genus *Piroplasma* may be distinguished from other intracorpuscular parasites by the fact of their commonly occurring in the form of paired pyriform bodies and less often in groups of four, eight, etc. within the infected corpuscles. The pyriform bodies arise by a peculiar budding process which they were the first to describe and figure (IV. 1907, p. 232). These characters suffice to distinguish the genus *Piroplasma* without entering into further details regarding the nuclear structure or mode of multiplication in the circulating blood. This process of multiplication has been observed repeatedly in *P. canis* (Nuttall and Graham-Smith, 1907) and *P. bovis* (Nuttall, 1909, see this volume, p. 341), in living parasites, and corresponding "budding" forms were detected in stained films of the three species of *Piroplasma* named.

Theiler (1904) was the first to demonstrate that *T. parva* is conveyed by ticks. He found that *Rhipicephalus appendiculatus* and *Rh. simus* were both capable of conveying East Coast Fever to healthy cattle. *Rhipicephalus appendiculatus*, having sucked infected blood as a larva is infective as a nymph, or having sucked infected blood as a nymph is infective in the adult stage. *Rh. simus*, infected as a larva, proved infective in the next or nymphal stage. Lounsbury (1906, p. 643) has found the adult infective. Theiler regards *Rh. appendiculatus* as the chief carrier of the disease. Further investigations by Lounsbury (1906) served to include *Rh. evertsi, nitens* and *capensis* among the pathogenic species: "In all cases the infection has been derived by the transmitting tick in one stage of its individual life cycle and communicated in the following stage."

Redwater and East Coast Fever may be said to resemble each other in that both diseases are conveyed by ticks, but whereas *P. bovis* persists in the blood for a considerable length of time after recovery and recovered or "salted" animals are capable of infecting ticks, the contrary holds for *T. parva*.

In short, there are ample grounds for referring the parasites of Redwater and East Coast Fever to different genera. We shall see that our studies upon the parasite, both living and in stained preparations, support the view that the parasites are very different.

Our observations were conducted upon the blood of two cows which were experimentally infected by means of ticks (*Rhipicephalus evertsi*, ♂s and ♀s) imported from South Africa. Nuttall and Graham-Smith (This *Journal*, p. 208) have reported the negative results of their attempts at cultivating the parasites present in the blood of these cows.

In both cases the parasites appeared at first in small numbers (5—6 %) and increased steadily in numbers until death when 60—75 % of the corpuscles of the peripheral blood were found to be infected.

The following protocols and charts relating to the two cows demonstrate the steady increase in the number of parasites on successive days.

Record of Cow 1.

Date	Day	Temp.°F.	
10. ii. 09.	1	—	30 ticks (*Rh. evertsi* ♂ s and ♀ s) were placed on the Cow.
	2	—	
	3	—	
	4	—	
	5	—	
	6	—	
	7	101·2 p.m.	
	8	102·2 ,,	
	9	101·6 ,,	
	10	101·4 ,,	
	11	101·8 ,,	
	12	—	
	13	102·0 ,,	
	14	102·0 ,,	
	15	103·0 ,,	
	16	103·0 a.m. 104·2 p.m.	
	17	103·2 a.m. 104·4 p.m.	Parasites first seen, very few.
	18	103·0 a.m.	,, very few.
	19	104·0 ,, 105·0 p.m.	5 % of corpuscles infected.
	20	105·6 ,,	10 % ,, ,,
	21	105·0 a.m. 105·4 p.m.	16 % ,, ,,
	22	105·0 ,,	25 % ,, ,,
	23	101·2 ,,	28 % ,, ,,
	24	103·6 ,,	40 % ,, ,,
	25	101·6 ,,	56 % ,, ,,
	26	101·4 a.m.	51 % ,, ,,
	27	104·6 p.m.	52 % ,, ,,
	28	104·6 a.m.	62 % ,, ,,
	29	105·4 ,,	62 % ,, ,,
	30	104·0 ,,	60 % ,, ,,
	31	102·0 ,,	75 % ,, ,,
13. iii. 09.	32	Cow died 7 a.m. of East Coast Fever.	74 % of corpuscles infected.

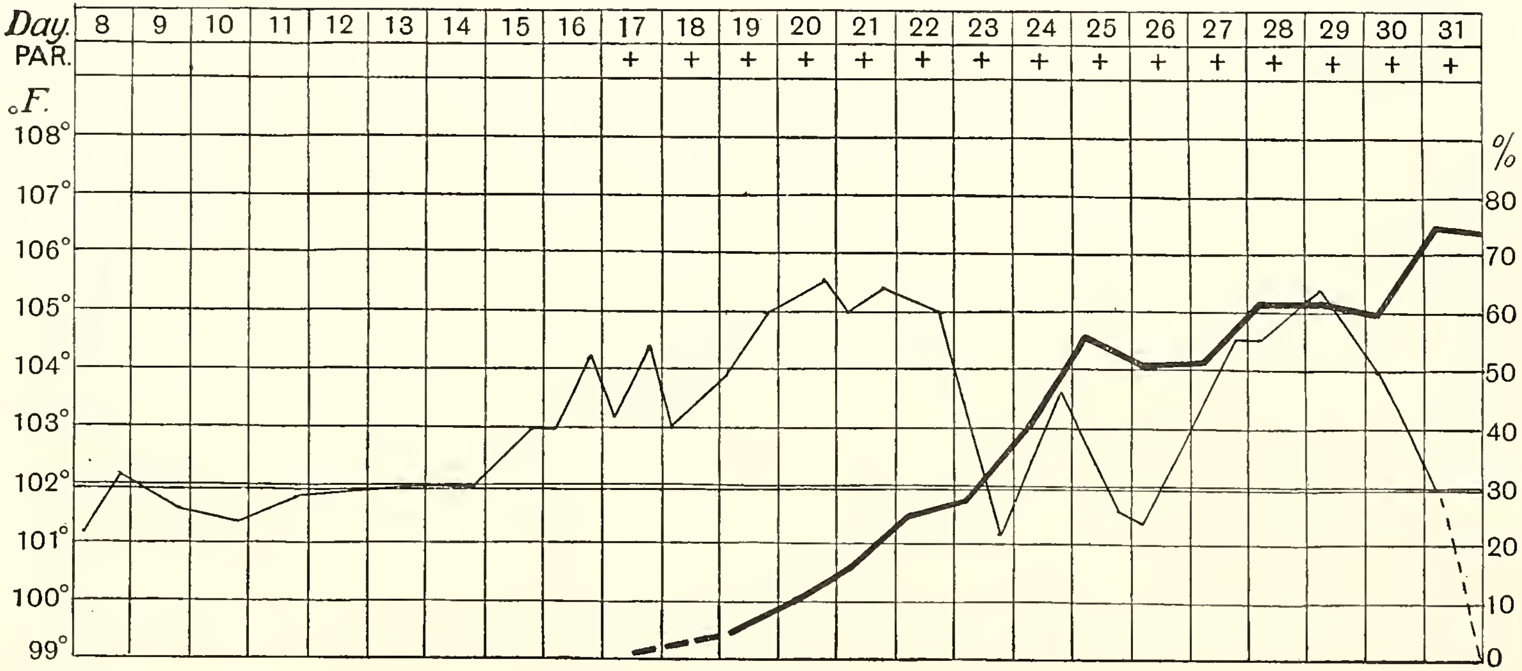
In the case of Cow 1 the temperature rose above 104° F. on the 16th day after the first ticks were applied, and the parasites appeared in small numbers on the 17th day. Beginning with the 19th day infected corpuscles occurred in appreciable numbers and their numbers increased steadily so that beginning with the 19th day 5 %, then 10 %, 16 %, 25 %, 28 %, 40 %, 56 %, 51 %, 52 %, 62 %, 62 %, 60 %, 75 % of infected corpuscles were recorded on successive days. In a blood film taken within a couple of hours after death on the 32nd day 74 % of the

Record of Cow 2.

Date	Day	Temp.° F.		
6. IV. 09.	1	101·0 p.m.	12 ticks (*Rh. evertsi* 2 ♂ and 10 ♀) were placed on the Cow.	
	2	101·4 ,,		
	3	101·4 ,,		
	4	— ,,		
	5	101·0 ,,		
	6	101·2 ,,	12 ♂ ticks placed on Cow.	
	7	101·0 ,,		
	8	101·0 a.m.		
	9	99·7 ,,		
	10	101·6 ,,		
	11	100·2 ,,		
	12	100·0 ,,		
	13	101·6 ,,		
	14	104·2 a.m. 103·8 p.m.		
	15	104·2 a.m. 105·8 p.m.		
	16	106·2 a.m. 107·0 p.m.		
	17	106·8 a.m. 106·8 p.m.		
	18	107·0 a.m.		
	19	105·8 ,,		
	20	105·0 ,,	Parasites first seen, few.	
	21	104·8 ,,	10 a.m. 6 % of corpuscles infected. 4 p.m. 8 % ,, ,,	*Treatment:* 200 c.c. 1 % Trypanblue.
	22	102·2 ,, 102·2 p.m.	5 ,, 8 % ,, ,,	200 c.c. 2 % Trypanblue.
	23	104·6 a.m.	10 a.m. 13 % ,, ,,	100 c.c. 2 % Trypanblue.
	24	103·6 ,,	10 ,, 22 % ,, ,,	
	25	102·6 ,,	10 ,, 26 % ,, ,,	
	26	102·8 ,,	10 ,, 32 % ,, ,,	
	27	104·0 ,,	10 ,, 41 % ,, ,,	
	28	105·0 ,,	10 ,, 47 % ,, ,,	*Treatment:* 100 c.c. Orsudan at 6 p.m.
	29	103·4 ,,	10 ,, 60 % ,, ,,	
5. V. 09.	30	—	9 a.m. Cow found dead of East Coast Fever. Highly tuberculous.	

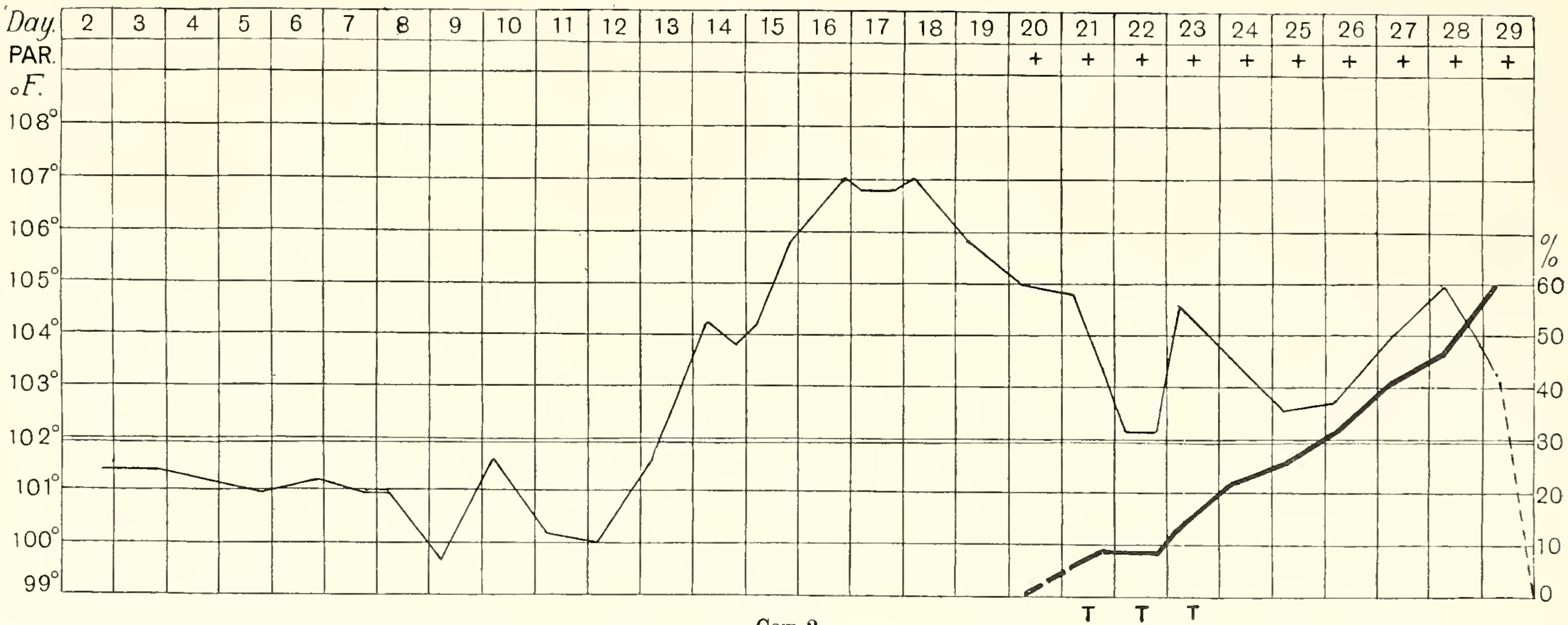
Note:—The drugs were injected intravenously (Trypanblue) and subcutaneously (Orsudan) in watery solution.

corpuscles were found to harbour parasites. In the case of Cow 2 the temperature rose above 104° F. on the 14th day after the first ticks were applied, and the first parasites were noted on the 20th day. On the morning of the 21st day 6 % of the corpuscles were found to harbour parasites and the number of infected corpuscles rose on successive days with remarkable uniformity: 8 %, 13 %, 22 %, 26 %, 32 %,



Cow 1.

Chart showing the body temperature beginning with the 8th day after the infected ticks were placed upon the cow. The parasites appeared on the 17th day. The increase in the percentage of infected red blood corpuscles is indicated by the heavy black line.



Cow 2.

Chart showing the body temperature beginning with the 2nd day after the infected ticks were placed upon the cow. The parasites appeared on the 20th day. The rise in the percentage of infected corpuscles is indicated by the heavy black line. The letters **T T T** beneath the chart indicate the three days on which Trypanblue was administered.

41 %, 47 %, 60 %, as is graphically shown in the chart. In both cases the initial rise of temperature was followed by a fall after which the temperature rose, fell, and rose again before falling prior to death. The number of infected corpuscles in the peripheral circulation in both cows is seen to bear no relation to the febrile reaction.

Cow 2 was subjected to treatment by one of us (G. H. F. N.) in conjunction with S. Hadwen, D.V.Sci., and the case has been briefly mentioned in another communication as proving that Trypanblue exerts no effect upon the parasites, and that it is consequently unavailing in the treatment of East Coast Fever. The administration of Orsudan on the 28th day likewise exerted no beneficial effect. It will, however, be noted that the injection of Trypanblue on days 21, 22 and 23 (indicated by T, T, T, below the Chart) may have influenced the temperature and slightly retarded the rise in the percentage of infected corpuscles—this may only be due, however, to a coincidence.

Observations upon the living parasite.

Our studies upon the living parasite have entailed a great amount of labour, and our results emphasize the difference between *Theileria parva* and *Piroplasma*. We have made innumerable observations upon fresh blood in films sealed with vaseline and as quickly as possible placed beneath the microscope. The technique was essentially similar to that used by Nuttall and Graham-Smith in their studies upon *P. canis*. The majority of the parasites were observed in films maintained at blood temperature in Nuttall microscope-thermostats, of which we had four at our disposal. The time occupied in the actual microscopic observation and sketching of living parasites amounted to 255·5 hours; this time was devoted to the observation of corpuscles containing single parasites (88·2 hours), two parasites (52·1 hours), three parasites (47·4 hours), four parasites (51·4 hours) and five to eight parasites (16·4 hours). The average time devoted to the continuous observation of 41 corpuscles containing single parasites was 129 minutes, of 21 corpuscles with two parasites was 149 minutes, of 14 corpuscles with three parasites was 203 minutes, of 15 corpuscles with four parasites was 205 minutes, of five corpuscles with five or more parasites was 196 minutes. Consequently, there was ample time devoted to the study of the living parasite. A considerable amount of the time was given to the observation of the largest single parasites which could be found in the hope that if division occurred it might the more readily be witnessed.

The movement of translation of the parasites within the corpuscles may be very active. In Diagram 1 A, A′, two observers (G. H. F. N. and G. S. G.–S.) recorded the movements of a single parasite which came under observation 22 minutes after the blood was drawn, the preparation being maintained at 37°C. Active movements continued up to 114 minutes, but they were not recorded longer than 94 minutes. Both

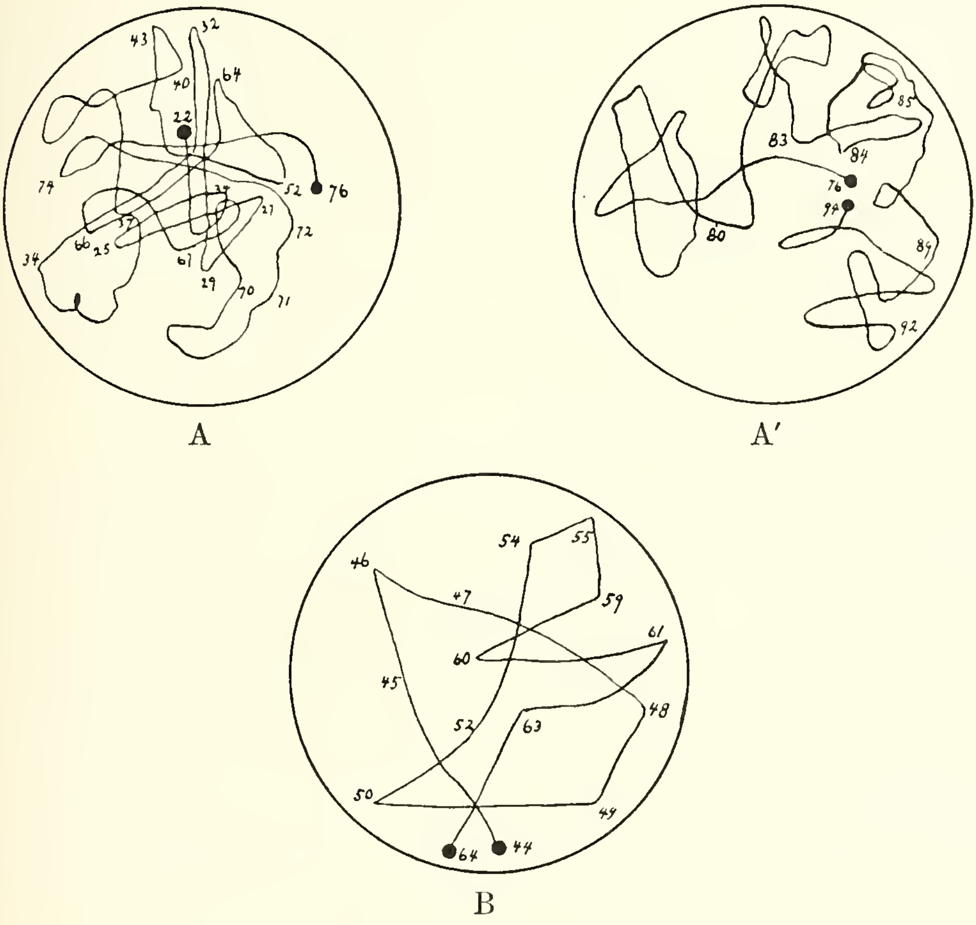


Diagram 1.

Fig. A and A′. Course of movement pursued by a single ovoid parasite during a period of 72 minutes. The observation commenced 22 minutes after the blood was drawn and ended at 94 minutes. The tracing A′ is merely a continuation of A; 28. II. 09. (G. H. F. N. and G. S. G.-S.)

Fig. B. Course pursued by a small pear-shaped parasite during the interval 44′ to 64′; 28. II. 09. (H. B. F.)

In both cases the observation was made at 37° C., and the observers agreed that the parasite appeared to grow slightly in size during the time they were observed.

N.B.—The numbers in the various diagrams denote the minutes that had elapsed since the blood was drawn and the time when the position of the parasite was recorded.

observers agreed that the parasite had apparently grown slightly in size during the interval. The parasite was ovoid in form throughout, except for a couple of minutes (52′—54′), when it appeared to protrude a bud-like process which grew in length and became narrower than the body of the parasite. The parasite then resembled a tetanus bacillus with a terminal spore (drumstick), but the process was soon retracted. In Diagram 1 B one observer (H. B. F.) recorded the movements of a small, single pear-shaped parasite during a period of 20 minutes (44′—64′). In both cases the parasite "explored" the interior of the corpuscle in all directions.

Similar observations were repeatedly made, but these figures will suffice to record the nature of the movement. Apart from "tacking about" at certain points the parasite occasionally rolled over. At times it moved with a fair degree of rapidity from one point to another, at other times it moved about slowly. The parasite was very distinctly seen as a rule, but occasionally it grew dim as in a fog owing, apparently, to the intervening haemoglobin, the parasite having wandered to the opposite side of the corpuscle.

The *alteration in form* observed in single parasites could be frequently attributed to their rolling over and being viewed in a different position. At other times we believed that what appeared to be processes were nothing but temporary creases in the corpuscular membrane giving rise to optical delusion. At other times, however, we were convinced that the parasites underwent actual alterations in form (Diagrams 2 and 3 A); the changes of form were slow and slight and not in any way to be compared to the active amoeboid movements of *Piroplasma canis* at certain stages of its development, as recorded by Nuttall and Graham-Smith. In most cases no distinct changes of form could be observed after prolonged observation lasting, in some cases, for hours. Similar appearances were seen in the case of corpuscles which contained two or more parasites. (Diagram 4.)

As in the case of *P. canis* and *P. bovis* no *structure* can be made out in the living parasite, and this appears natural in view of the extremely small size of the organism.

In no case could we satisfy ourselves that intra-corpuscular parasites underwent *multiplication*. What appeared to be division was, indeed, repeatedly observed (Diagram 3, Series B and C). The parasite assumed a double pyriform shape, the swollen portions (B 31′, C 217′) remaining connected by a strand of protoplasm. Such forms were at times observed for several hours, but no further change of form took place, although the parasite frequently altered its position within the corpuscle. Similar forms are encountered in stained preparations. In two cases, one of which we illustrate (Diagram 3 B), two parasites appeared to be formed and to separate, but they may have remained connected by an invisible band of protoplasm. That such appearances are very deceptive has already been noted by Nuttall and Graham-Smith in the case of *P. canis*, and the observation (one of two) recorded in Diagram 3 C also indicates the need of caution in interpreting appearances when dealing with the still more minute parasites of East Coast Fever. Thus, in the figure referred to, the two connected pear-shaped portions of an apparently dividing parasite, formed a single mass

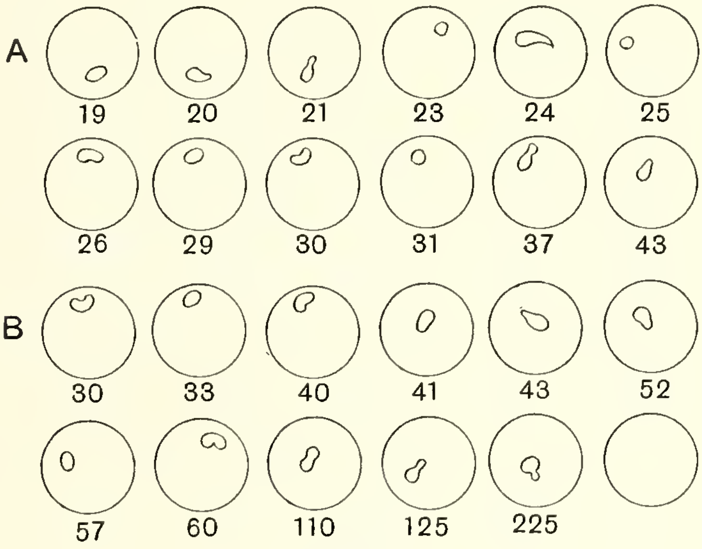


Diagram 2.

Fig. A. Showing the movements and changes of form of a single intra-corpuscular parasite during the period 19′—43′. Observation at 37° C.; 2. III. 09. (G. H. F. N.)
Fig. B. Similar to A. Period of observation 30′—225′, at 38° C.; 12. II. 09. (A. P.)

when the corpuscle vanished (372′), only a small particle being seen to lie beside it. To have satisfied ourselves that actual division had taken place we should have observed a wide separation of the supposed daughter cells, but this we were never able to do, neither could we observe the escape of one of the newly-formed parasites from the infected corpuscle, as Nuttall and Graham-Smith were able to see in *P. canis*. It is possible, however, that what we have figured at C 265′ may represent an early stage of division, and that, if the corpuscle had vanished at a later stage, two separate parasites might have been seen. Again and again we kept apparently dividing forms under observation with doubtful results; the observation only ended when the corpuscles became crenated or the parasites faded away after the corpuscle had vanished. At times the stems of the pears and the two swollen portions appeared to be connected by a delicate web of protoplasm; at other times, when rotated, the parasite appeared like an 8 (compare C 102′, 131′, 217′). On the other hand, hour glass or 8-forms and bacilliform parasites have repeatedly been observed to persist unchanged for hours. Similar observations were made on corpuscles containing what appeared to be 2, 4, 5, 8 parasites. (Diagram 4.)

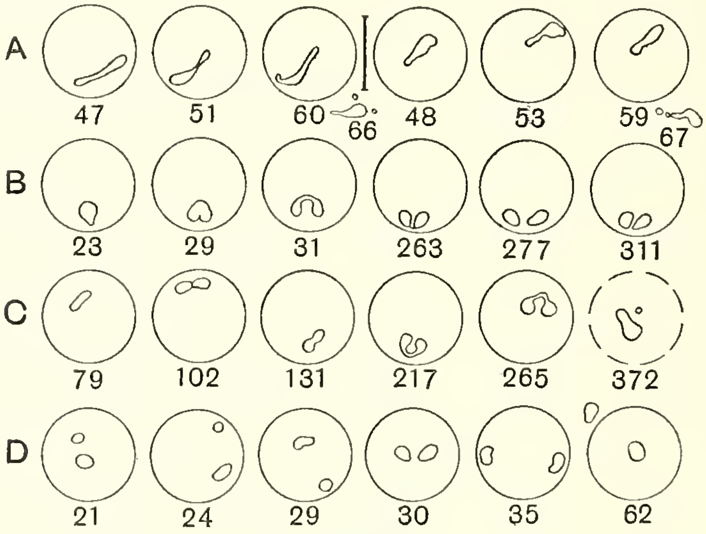


Diagram 3.

Fig. A. Illustrates the changes of form of bacilliform parasites, the first observed during the period 47′—66′, the second during the period 48′—67′. The corpuscles vanished in both cases at the end of the observation and the parasites broke up rapidly into bead-like particles which ultimately disappeared. Observation at 42° C.; 6. III. 09. (G. H. F. N.)

Fig. B. Illustrates the apparent division of a parasite into two. Observation at 38° C.; 3. III. 09. (A. P.)

Fig. C. Illustrates the apparent division of a parasite into two, but when the corpuscle vanished (372′) but one parasite (and a fragment?) was left which then degenerated. Observation at 37° C.; 4. III. 09. (G. H. F. N. and H. B. F.)

Fig. D. Selected figures from a series, illustrating the movements of two parasites within a corpuscle and the escape (62′) of one of the parasites from the corpuscle which remained uninjured and retained the other parasite. Observation at 10—12·5° C.; 28. II. 09. (G. H. F. N. and H. B. F.)

Growth: In several instances two observers agreed that the parasite had increased slightly in size in the course of 1 to 2 hours. It was also noted that intracorpuscular parasites seemed at times to disappear completely.

Appearances seen in vanished corpuscles: When, as occasionally occurs, the corpuscles lose their haemoglobin or "vanish" the parasites may be distinctly seen within the circular contour of the "ghost" of the corpuscle. When the corpuscles vanish, the parasites rapidly lose their shape and undergo degeneration, small spherical masses of protoplasm being formed from the substance of the parasite, which ultimately vanishes (Diagram 3 A). Two or more separate parasites may thus be distinctly seen within the outline of a vanished corpuscle. At times the clearer view obtained of the parasites in a vanished corpuscle reveals the bands uniting parasites which previously appeared to be discrete (compare Diagram 4 D, 134′ and 250′).

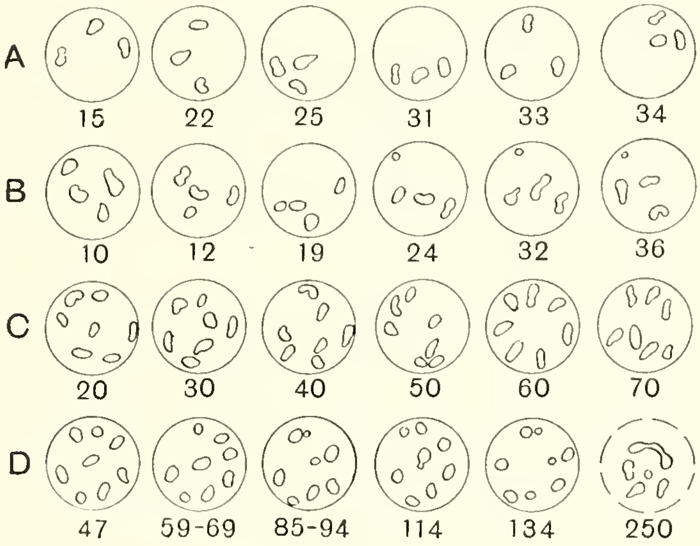


Diagram 4.

Fig. A. Selected series of figures illustrating the movements of three intracorpuscular parasites during the period 15′—34′. The parasites were kept under observation for 279 minutes, during which period they remained active, but no multiplication took place. Observation at 43° C.; 8. iii. 09. (A. P.)

Fig. B. Selected series of figures, etc., of four parasites 10′—36′. No change had occurred after 7 hours 40 minutes. Observation at 15—16° C.; 6. iii. 09. (H. B. F.)

Fig. C. Selected series of figures, etc., of seven parasites, 20′—70′. Although the observation continued 2 hours no result could be recorded. Observation at 42° C.; 9. iii. 09. (A. P.)

Fig. D. Selected series of figures, etc., of "8 parasites," 47′—250′. The corpuscle vanished in the end (250′), and but 5 parasites could be counted. Observation at 42 °C.; 6. iii. 09. (H. B. F.)

The escape of parasites from infected corpuscles was recorded on six occasions only. In no case did any injury of the corpuscle accompany the escape of the parasite. In three instances we were dealing with a singly infected corpuscle, in two instances with a doubly infected corpuscle, in one instance the corpuscle contained four parasites. In the cases of the two doubly infected corpuscles both parasites escaped from the one corpuscle and only one parasite escaped in the other instance. From the corpuscle containing four parasites two of the latter were "shot out" at opposite sides of the corpuscle. All three of us had an opportunity of observing the escape of parasites from corpuscles.

On the other hand, only one of us (A. P.) has seen *parasites escape and re-enter* fresh corpuscles; the observation was made on but two occasions. In the first instance (Cow 1, 20th day, 10 °/₀ of r.b.c. infected) an ovoid parasite escaped and immediately indented and re-entered a fresh corpuscle, 24 minutes after the blood had been drawn (observation at 37° C.). In the second instance (Cow 1, 21st day, 16 °/₀ of r.b.c. infected) a rounded parasite escaped 13 minutes after the blood was drawn and after zigzagging about in the serum for 15 minutes it penetrated a fresh corpuscle; although observed, subsequently, for nearly an hour no further development followed.

CONCLUSIONS AND SUMMARY.

The mere fact that East Coast Fever is not communicable by blood inoculation indicates that the infection does not occur through the escape of parasites from infected corpuscles and their entry into fresh corpuscles, as in true piroplasmosis. Judging, however, from two observations made by one of us (see previous section) the parasites may at times escape and re-enter corpuscles. How are these conflicting statements to be brought into accord? If it is true that the escaping parasites actually re-enter fresh corpuscles under natural conditions, then the non-communicability of the disease can only be due to the parasites being incapable of further multiplication in the freshly invaded corpuscles, and the phenomenon of their escape and re-entry is without significance either in relation to the disease or to the biology of the parasite. The question then arises: How do the corpuscles become infected?

Although the significance of "Koch's bodies" in the spleen and other internal organs of animals infected with East Coast Fever is still obscure, one of us (G. H. F. N.), acting on the assumption that they may bear a causal relationship to the disease, inoculated a fresh emulsion of infected spleen and bone marrow subcutaneously into a fresh animal. The result was negative[1]. The experiment was also dictated by the belief that the invasion of the corpuscles may take place in the spleen or bone marrow and that the corpuscles act chiefly as vehicles (after the manner of an omnibus) for parasites destined solely to gain an entry into ticks for the purpose of undergoing further development. On the "omnibus hypothesis" the corpuscles are penetrated by parasites as the blood circulates through the internal organs; the parasites multiply in these organs but not in the corpuscles. As the disease progresses more and more protozoal passengers board the transporting vehicles, the corpuscles. This hypothesis offers an explanation of the fact that (1) the disease is not communicable by blood inoculation; (2) that the percentage of infected corpuscles not only increases steadily, but that the percentage of corpuscles containing many parasites grows as the disease advances; (3) that the parasites do not persist in the blood after recovery. It is, of course, conceivable that intracorpuscular multiplication may take place; if it occurs the process must be very slow.

The assumption appears justified that the parasites obtain nutriment from the corpuscles they inhabit. The discovery recently made by Meyer that East Coast Fever may be communicated by transplantation of the affected spleen or pieces of that organ goes to support the hypothesis here advanced, for doubtless the transplanted splenic tissue lived on for a time in the peritoneal cavity of the experimental animals and served as a nidus wherein the parasites continued to multiply. Meyer's experiments demonstrate that the spleen, and possibly other internal organs, serve as the primary seats of the invasion of the blood corpuscles by the parasites.

Summarizing our observations on the living parasite, we would state that they show active movements within the corpuscles and at times undergo alterations of form. No structural details can be made out in living parasites. We have not obtained any conclusive evidence that the parasites multiply within the infected corpuscles, but at times appearances were observed suggesting this possibility. In several cases the parasites seemed to grow slightly in size during the period of observation. When infected corpuscles lost their haemoglobin the parasites were distinctly seen for a time, after which they degenerated and died. The escape of parasites from infected corpuscles was witnessed on six occasions; it was accomplished without injury to the corpuscle. On two occasions the escaped parasites appeared to re-enter fresh corpuscles.

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[1] Koch and his colleagues have recorded similar results following the inoculations of pulped spleen and lymphatic glands. Mr S. Stockman, M.R.C.V.S., informs us that he and Mr A. Theiler, working in the Transvaal, likewise obtained negative results from inoculations with spleen emulsions. Meyer (IX. 1909, Hague Congress of Veterinary Medicine) has recently succeeded, however, in communicating the disease by transplanting the whole spleen or portions of infected spleen into the peritoneal cavity of healthy animals.

Our studies on *T. parva* in stained films will shortly follow.

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NOTE ON THE MODE OF MULTIPLICATION OF *PIROPLASMA BOVIS* AS OBSERVED IN THE LIVING PARASITE.

By GEORGE H. F. NUTTALL, F.R.S.

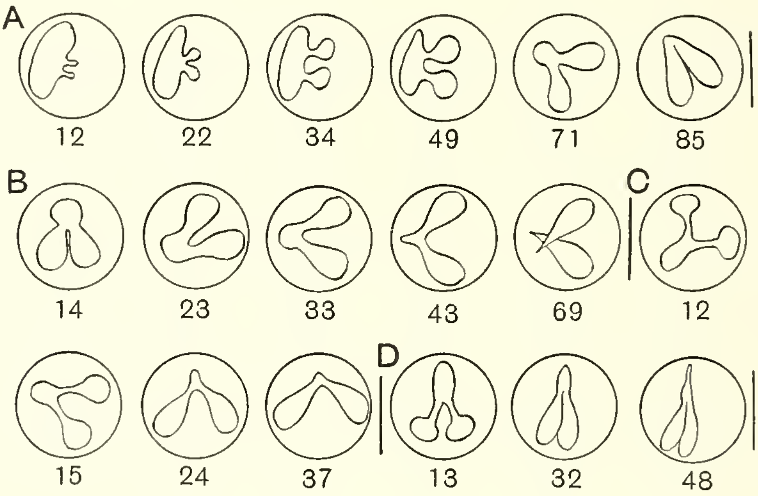
(One diagram.)

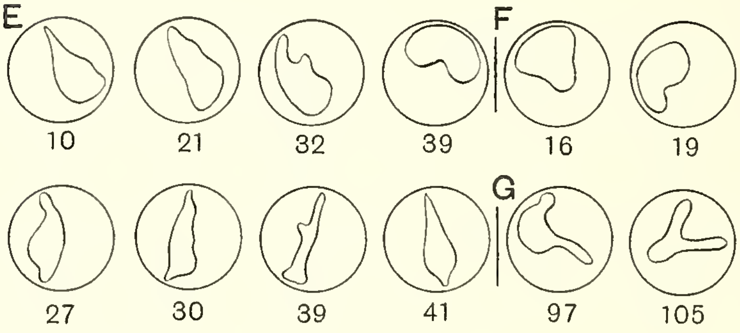
In a previous paper, Nuttall and Graham-Smith (*Parasitology*, 1908, vol. I. p. 134) described the appearances presented by *P. bovis* in stained films, and showed that they agreed with those of *P. canis* and *P. pitheci* with regard to the presence of the "budding" or multiplication forms. The multiplication of *P. bovis* in fresh blood films remained, however, to be described.

Whilst conducting experiments on the treatment of cattle with trypanblue (Nuttall and Hadwen, *Parasitology*, 1909, vol. II. p. 236), I had an opportunity of studying the living parasite in films prepared from ear-vein blood of certain of our control animals. The films were prepared in the usual way, sealed with vaseline and as rapidly as possible placed in a Nuttall microscope-thermostat.

Certain facts regarding the parasite have already been noted (*Parasitology*, vol. II. p. 239): the existence of many large, single, ovoid or pyriform parasites differing from those observed in *P. canis* and a much higher percentage of dividing forms than is encountered in blood containing the canine parasite. It was also noted that *P. bovis* shows less active movement than *P. canis* in the amoeboid stage. Whereas, usually, a maximum of 5—6 % of dividing forms are encountered in the case of *P. canis*, no less than 13—16 % of dividing forms may be seen in the case of *P. bovis* in some animals at certain stages of the disease.

The accompanying diagram illustrates the mode of division of *P. bovis* and the character of its amoeboid movement as observed in fresh blood. The figures are selected from a series of sketches made whilst watching the living parasite under the microscope. The letters A to G denote as many different parasites. Thus, parasite A is represented by six figures, the numbers 12, 22, 34, etc. beneath the figures denote the minutes that had elapsed from the time the blood was drawn to the time when the particular sketch was made. The time was recorded by means of a stop-watch, which was started at the moment when the sample of blood was taken from the animal's ear-vein.





Figures A to D represents the process of division as observed in four different parasites. Parasites A, B (and G) were observed at 41° C. in one preparation and in the same field. The blood was derived from Cow I (this vol. p. 252) on the 5th day after inoculation. The cow was immediately afterwards treated with trypanblue and recovered. At the time the blood-sample was taken, ·8 % of the red blood corpuscles harboured parasites, and 4 % of these were represented by dividing forms. The presence of so many parasitized corpuscles in one field (there were several more besides) is explained by the tendency infected corpuscles have to become aggregated into groups. In parasite A the commencement of the "budding" process is seen at 12 minutes, and, after the expiration of 85 minutes the parasites are almost completely formed but remain joined. In parasite B the first drawing was made at 14 minutes (at a later stage than the first stages of A) and, after 69 minutes, we see two clearly separated pyriform parasites lying with their points crossed within the corpuscle. Whereas, in the early stages of division, the "buds" protruded laterally in parasite A, they protruded at one end of the oblong parasites C and D. The latter was observed at 36° C. in a sample of blood taken from Control Cow I (this vol. p. 244) on the 8th day after inoculation. The blood was examined on two occasions on the same day at times when the number of infected corpuscles amounted to 3·2 °/₀ and 1·2 °/₀ respectively, and the corresponding proportions of dividing forms were 2 °/₀ and 3 °/₀. Parasite D rotated upon its long axis as division proceeded, consequently the two parasites which were being formed appear slightly superposed. But for the fact that the process of multiplication is slower, it appears in all respects identical with what we have observed in *P. canis*.

Figures E to G represent the character of the amoeboid motion, which is usually not at all marked. Parasite E was observed in the blood of Cow X (p. 242) at a time when ·8 °/₀ of the corpuscles were infected, that being the maximum percentage of infection attained. Parasite F was observed on the succeeding day in the blood of the same animal when the number of infected corpuscles had fallen to 0·1 °/₀. Parasite G was sketched at the same time as A and B; it showed more marked pseudopodia than usual, but the movements were not to be compared in point of rapidity with those of *P. canis*.

A NOTE ON THE SPIROCHAETE PRESENT IN ULCERATIVE GRANULOMA OF THE PUDENDA OF AUSTRALIAN NATIVES.

By W. CECIL BOSANQUET, M.A., M.D., F.R.C.P.

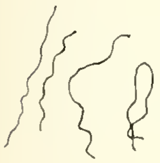
(From Prof. E. A. Minchin's Laboratory of Protozoology, Lister Institute of Preventive Medicine.)

(Five Text-figures.)

Early in January of the present year Professor Minchin kindly gave me for examination a portion of tissue and a mounted section taken from a case of the above disease and sent him by Dr J. Burton Cleland, then of Perth, W. Australia. The tissue was already embedded in paraffin, and both it and the section were stained by Levaditi's method and showed the presence of numerous spirochaetes.

These organisms were situated deeply in the tissue, 3 or 4 mm. from the surface, in the fibrous layer forming the base of the ulcer, and were most numerous in the immediate neighbourhood of the blood-vessels of this part. A few were visible within the vessels themselves. The longest forms seen measured on an average 12μ in length: they were so slender that their breadth was rather a matter of guess-work, but it may perhaps be put at $\frac{1}{4}\mu$ (Fig. 1). Shorter forms were common, ranging down to 3 or 4μ in length: these or some of them may have been due to division of longer forms in cutting sections of the tissue. There were a few individual organisms which were somewhat thicker than the average ($? \frac{1}{2}\mu$).

The spirochaetes were in some instances homogeneous throughout in appearance, but a considerable number presented at certain points in their length definite granules staining more deeply than the rest of the protoplasm (Fig. 2). In some cases these granules were distinctly greater in diameter than the rest of the organism. Terminal granules of this latter kind were not uncommon: a few spirochaetes were seen possessing two well-marked granules near to one end (Fig. 3). In some specimens the whole protoplasm was granular throughout, so that the organism resembled a string of beads closely threaded: these were presumably degenerative forms. A few instances of abnormal forms were also seen—*e.g.*, a thick specimen with a well-marked loop or vacuole near to one extremity; a second in which a large terminal granule present was transparent and unstained, giving somewhat the appearance of a loop; and a few individuals coiled up into rings (Fig. 4). I found one or two instances of what appeared to be forked forms, suggesting longitudinal division, and Cleland also saw a few of these; but I do not feel at all certain that these specimens were really bifid, as it is difficult to eliminate possibilities of mere apposition of organisms when dealing with creatures of such tenuity.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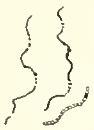
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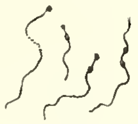
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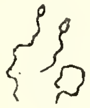
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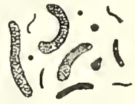
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The curls or waves in the spirochaetes were quite irregular, unlike the symmetrical curls of *Treponema pallidum.* Some organisms lay almost in straight lines: others were twisted on themselves, and others again were merely sinuous (Fig. 1). On the whole they resembled very closely the figures given by Castellani of his *Spirochaeta pertenuis* of yaws, though the illustrations of this parasite given by Levaditi and Nattan-Larrier make it closely resemble *T. pallidum.* In a piece of yaws-granuloma very kindly sent me by Dr Castellani, I was unsuccessful in finding the parasites by the silver-method, as I had hoped to do for the sake of comparison. The spirochaetes in the present case of ulcerative granuloma did not resemble, except in slenderness, the illustrations given by Maclennan of this organism: possibly those from which his figures are taken were derived from the surface of the ulcers and may have assumed a more curved appearance, when thus lying free, than they present when compressed into the interstices of the tissues. No illustrations are given by Wise who first discovered spirochaetes in this disease. He originally found them in smears from the diseased tissues—some of them taken from the deeper parts. He does not, however, speak of having stained the organisms in sections of the tissue.

So far as I am aware, Cleland is the first to supply this proof of the connexion of spirochaetes with the malady.

In the specimens which I examined, the spirochaetes were accompanied by large numbers of bacterial forms. These were found wherever the spirochaetes lay, and were absent where the latter were absent. The bacteria were very irregular in shape and size, ranging from granules about $\frac{1}{2}$—1μ in diameter and slender rods (2 or 3μ by $\frac{1}{2}\mu$) to large rod-shaped organisms, often pretty sharply curved, some 6μ in length and about 1μ in thickness (Fig. 5). The question of the relationship of the bacteria to the spirochaetes is of importance in connexion with that of the aetiology of the disease. Both organisms lay deeply in the tissues and were present in large numbers. The bacteria did not resemble, so far as I could judge, any of the known agents of infective disease: at the same time it seems unlikely that mere saprophytes could establish themselves in such numbers at a point so far from the surface. Hence there is a probability that either the spirochaetes or the bacteria are the causal agents of the ulceration. If this be so, the coexistence of the two forms might be explained either as an example of symbiosis or on the ground that they represent different stages in the development of the same organism. On the latter hypothesis transitional stages might be seen either in the small slender bacterial forms, corresponding as they do with the spirochaetes in thickness and possibly representing the results of transverse division, or in the spherical granules which resemble bacterial spores and may be the granules seen at the extremities and occasionally in the substance of the spirochaetes.

In this connexion it is interesting to recall that in Vincent's angina fusiform bacilli and spirochaetes invariably coexist, and that Ruth Tunnicliffe believed that the latter developed in cultures of the former; that rod-like bodies were found along with spirochaetes on the surfaces of ulcerated cancers by Hoffmann and by Loewenthal; that bacteria and spirochaetes were found together in onychia by Dutton, Todd and Tobey; that Levaditi states that vibrio-forms develop from spirilla in E. African tick-fever; and that Castellani in yaws and Leuriaux and v. Geets in syphilis found oval forms, which the latter authors regard as spores from which the *Treponema* develops. In several of these cases, the question of symbiosis or of varying forms of the same organism is undecided. Markham Carter regards the bacteria present along with the parasites (*Helcosoma tropicum*) in Oriental Sore as symbiotic. The need for such partnership might constitute one of the difficulties in cultivating spirochaetes, which have so far proved practically insuperable. Cleland cultivated bacteria and cocci from the ulcers, but failed to inoculate animals with either of them. He was equally unsuccessful with inoculations of material taken directly from the sore. He has named the spirochaetes of ulcerative granuloma *Spirochaeta aboriginalis* as affecting the aborigines of Australia.

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THE MORPHOLOGY AND LIFE-HISTORY OF *CRITHIDIA GERRIDIS*, AS FOUND IN THE BRITISH WATER-BUG, *GERRIS PALUDUM*.

By ANNIE PORTER, B.Sc. Lond.

(With Plate IV.)

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Introduction.

One of the most noteworthy advances made in medicine in recent times is the recognition of the part played by insects and other *Arthropoda* in the spread of disease. It is only necessary to recall the connexion of malaria with mosquitoes, of human relapsing fevers with *Ornithodoros moubata,* and of sleeping sickness with the tse-tse fly, to recognise the enormous importance of the economic side of protozoology in connection with *Arthropoda.*

Unfortunately, in the desire to fill up the many gaps in our knowledge of the life-histories of disease-causing agents such as *Trypanosoma gambiense*, the occurrence of natural parasites of the fly or other arthropod has sometimes been overlooked, and as a result of this, phases of the life-history of probably harmless parasites of insects have been mistaken for those of the deadlier organism, acquired by the host more or less casually in its food, and later transferred, also casually, to some vertebrate host.

The study of the natural flagellates of arthropods therefore is most important, and it is necessary to study both the distribution of the host and of the flagellate it harbours.

The subject of this paper is a flagellate, *Crithidia* allied to *Trypanosoma*, occurring in *Gerris paludum*[1], a common British water-bug, popularly known as the "pond skater." A nearly allied species of *Gerris*, *G. fossarum*, common in Madras, also harbours a *Crithidia*, and the similarity between *C. gerridis* as described by Patton (1908) and the parasite that I have found in *G. paludum* is such that it is impossible to consider it other than the same organism. As the host occurs in a non-tropical region, there are slight differences between its parasite and that of the Indian form, so that I propose to describe, in detail, *C. gerridis*, occurring in a British species of *Gerris*.

MATERIALS AND METHODS.

The *Gerris paludum* used in this investigation were collected from many localities in the south and east of England. Thus, specimens of the insect were examined from many places in Sussex, Middlesex, Surrey, Bucks, Gloucestershire, and around Cambridge, and *Crithidia* were found in the water-bugs from every one of these districts.

During this investigation all stages of *Gerris paludum*, from very young nymphs to adults, and also a large number of embryos were examined, but while many of the young nymphs were found to be infected, I never found an infected embryo.

The *Gerris* were carefully dissected, and the unruptured gut was divided rapidly into crop, stomach, intestine, and rectal portions, each portion being teased in 0·8 % NaCl solution. Preparations of the Malpighian tubes and ovaries were also made, and the faeces of the bug were examined. For observation of the living organisms, wet preparations were covered and the edge of the coverglass was surrounded by vaseline, or similar preparations with wax feet which were then vaselined at the edges were made, while hanging drop preparations were also used. Methylene blue and neutral red were sometimes used as *intra vitam* stains but were not found of much value.

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[1] I have much pleasure in thanking Mr W. L. Distant for kindly identifying the species of *Gerris* for me.

For staining purposes, freshly teased material was fixed wet with formalin vapour or osmic acid, then transferred to methyl or ethyl alcohol and finally stained. Many preparations were mounted in Canada balsam, and such preparations were found of greater service than the ordinary uncovered films. Some preparations were also fixed wet with Bouin's fluid (picro-formol-acetic) and with Schaudinn's fluid (corrosive-acetic-alcohol).

For purposes of comparison several stains were used. Giemsa's modification of the Romanowsky stain was very serviceable, though it has the effect here as elsewhere of intensifying the chromatin. The procedure often adopted was to fix the wet preparation with osmic acid followed by absolute alcohol, transfer it to 50 °/₀ spirit and grade it down to water. The film was then stained with Giemsa's stain, rapidly dehydrated with acetone and then mounted in Canada balsam in the usual manner. Gentian violet also was used to stain "wet" preparations, and was found specially valuable in determining the structure of the membrane, while Delafield's haematoxylin was of much use for the same purpose. Thionin preparations and some stained with iron haematoxylin followed by orange-G were also utilised, and the results obtained by each method were carefully compared.

OCCURRENCE OF THE PARASITE IN THE HOST.

In a heavily infected specimen of *Gerris paludum* the *Crithidia*, in various stages, are found throughout the alimentary tract and in the faeces. Early stages of the *Crithidia*, the pre-flagellate stages, are the most abundant in the crop. The dilated stomach and intestine that follow, are often almost entirely blocked by the masses of flagellated forms. On one or two occasions, I have seen a few parasites in the Malpighian tubes, but this situation is not a common one for them.

The rectum of the bugs contains post-flagellate or encysted forms adapted for resisting desiccation outside the body of the host, as well as free flagellates that probably perish. Both these forms are found in the blackish faeces voided by the *Gerris*.

In a large percentage of the adult female *Gerris* examined, parasites have been found in the ovaries, and I have seen parasites pass out from an unruptured gut at that part of the gut adjacent to the ovaries, and make their way into the latter organs. Though very careful search has been made, the passage of the flagellate into the ova has never been seen. I may add that *Crithidia* are often less abundant in male *Gerris* than in females.

GENERAL DESCRIPTION.

The adult *Crithidia* are flagellates each possessing a long, narrow, tapering, fusiform body, which has its ectoplasm or periplast produced outwards as a thin, narrow membrane, whose edge, thickened as a chromatic border, passes on as the flagellum (Pl. IV, Figs. 21—46). In life, the flagellum and membrane can be readily seen, the lashing movements of the former making it more conspicuous.

The adult parasites measure from 16μ to 80μ in total length[1] and are 2μ to 4μ broad, while the flagellum from its basal granule to its tip may be 8μ to 40μ long. The flagellum even in life is seen gradually to taper from its origin to its free extremity. As the organism moves with its flagellum forwardly directed, this end of the organism is the anterior end.

The nucleus of the parasite in life appears as a bright, refractile spot. It is usually oval in contour. Near to it a much more highly refractile, rod-like body, the blepharoplast is seen. Other granules, also having great refringency, are scattered through the general body substance. Staining reactions show that some of these granules are composed of chromatin and so are chromidia (Pl. IV, Figs. 28, 34).

The organism has a richly granular, highly alveolar endoplasm (Figs. 26, 28, 44), and very occasionally in life the granules are so arranged that a clear central space is left somewhat suggestive of a cytopharnyx (Fig. 31) such as Léger mentions in *Herpetomonas jaculum*. But such cases are few, and as there is no evidence of the food-assimilating nature of such a space, I think it would be better to consider this arrangement as a more or less unusual, rather than as a normal, feature of the cell.

Stained preparations show the features mentioned before more clearly. They also show that the ectoplasm is thin and that the end of the body near the flagellum contains but little endoplasm in many cases.

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[1] This total length is measured from the extreme free end of the flagellum to the extreme posterior end of the body. There is much variation in the length of the body, but the flagellum is always about half the total length of the body.

The pre-flagellate and post-flagellate stages of the parasite will be described fully in subsequent sections. These stages have a general resemblance to the Leishman-Donovan bodies of Kala-azar.

MOVEMENTS.

The movements of the adult flagellate are very rapid but can be studied with comparative ease when the parasite is enclosed within a limited space by débris present in the gut.

In forward progression, the flagellum is anterior and the undulating membrane can be seen, its chromatic (flagellar) border appearing as a bright line. The membrane is truly undulatory, crests and troughs of waves appearing along it. As the organism moves forwards, the flagellum executes spiral, lashing movements and waves pass down it and the membrane to the posterior end of the body. The effect is to propel the flagellate forwards. At the same time that the organism moves forwards, it also rolls slightly from side to side. This movement has been noticed by Patton (1907) in *C. gerridis* from *Gerris fossarum*, *Microvelia* and *Perittopus*.

When the movements are very rapid, the anterior part of the body also seems to take some share in the lashing movement and the posterior region of the body has been seen in violent motion when the parasite had become attached by its flagellum to some débris and was endeavouring to free itself therefrom. The parasite may attach itself at times to the gut-wall of the host by means of the flagellum.

Reversal of the direction of motion takes place very rapidly and with ease. The flagellum curves gradually in the direction required and the parasite moves on a seemingly parabolic path till its body is almost parallel to its first position. The flagellum which initiates the reversal of direction at times lies quite close to the body. The latter then suddenly swings through an angle of 180° and lies almost in a line with the flagellum. The parabolic path is not nearly so obvious in this case.

Now and again I have watched parasites among débris. They have been slightly fixed at the posterior end while the anterior end has been free. The whole organism moved actively, its free end rotating through 360° in less than a second, the attached end acting as a pivot about which rotation occurred.

This movement would appear not to be a common one, but due to the position of the organism at the time.

Very frequently great clusters of *C. gerridis* (Pl. IV, Fig. 47) occur when the gut contents are placed on a slide. The parasites then are very conspicuous and the combined movements of them recall the movements of ciliated epithelium. As a rule, each *Crithidia* has its own independent motion. It is worthy of note that the shimmering appearance of the groups is due to the twisting movements of the *bodies* of the parasites, for in these aggregations the flagella are towards the centre of the group and serve to attach the organisms to the anchoring cells or débris, and so there is little or no flagellar motion. Parasites exhibiting torsion of the body are shown in Figs. 30 and 54, also some in Fig. 47.

Often the organisms seem to swell out and thicken in the centre, a somewhat "peg-top" like appearance resulting. This is not easily seen in fixed smear preparations though occasionally it is found (Pl. IV, Figs. 23, 38).

At times, forms are seen in which the posterior end of the body has contracted considerably so that the membrane appears of relatively greater length than in ordinary specimens. At the same time the membrane moves violently, being thrown into large waves along the body. The *Crithidia* then has an appearance very like that of a trypanosome, but if conditions of light and temperature become more favourable, normal movements are resumed, the resemblance to a Trypanosome disappears, and one realises that the seeming Trypanosome is only a *Crithidia* moving in an aberrant fashion.

MORPHOLOGY.

The life of the *Crithidia* may be considered as having three phases: (1) a stage in which no flagellum is obvious as such, but in which a flagellum gradually develops—the pre-flagellate stage; (2) a fully flagellated stage; (3) a form specially adapted for resisting desiccation, possessing no flagellum, but existing as a cyst—the post-flagellate stage.

The Pre-flagellate Stage.

This phase of the parasite is best seen in the nymphs of *Gerris paludum*. When the crop of an infected nymph is examined, small, oval or pear-shaped bodies, like Leishman-Donovan bodies, are found in it (Pl. IV, Figs. 1—4). Occasionally they are somewhat quadrilateral in form. They have no flagellum. These are the early pre-flagellate forms of the parasite. They are from 3μ to 7μ long by 2μ to 4μ broad. Their ectoplasm is slight and the endoplasm is richly granular. The nucleus lies often to one side, and is usually oval (Figs. 1—7). Darker staining chromatin granules are present in the nucleus. The darkly staining blepharoplast is usually rod-shaped (Figs. 1—4) but occasionally appears round (Fig. 13). It is situated near the periphery, often away from the nucleus and may lie somewhat transversely to the long axis of the pre-flagellate (Figs. 1, 2, 6). Often the outline of the blepharoplast is entire, but in forms about to divide, it may show a constriction in the middle (Fig. 16). The future flagellum appears as an area staining pinkish with Giemsa (Fig. 7) or deeply blue with Delafield, lying near the nucleus and the blepharoplast. This area next becomes somewhat rod-like and finally grows out as a delicate thread (which readily takes up chromatin stains) forming the flagellum (Figs. 8—11). The periplast, the somewhat rigid, condensed ectoplasm, of the body remains in close contact with the flagellum and becomes drawn out with it, forming a fine narrow membrane (Fig. 19). At first, the flagellum lies near the edge of the body, in fact usually touching it (Figs. 8, 9, 10) but as growth proceeds, it gradually becomes free at the distal end (Figs. 11, 12, 13, 14), but the fine membrane connects it to the body for at least half its length.

Longitudinal division (Figs. 16—20) occurs in the pre-flagellate forms as well as in those possessing flagella. As the process is much the same in both forms, it will be described in dealing with division.

The Flagellate Stage.

The general structure of the fully developed flagellate has already been indicated.

The long gregariniform body of the *Crithidia* has a very definite outline (Figs. 21—46). It is not rigid, however, but elastic and capable of active movements. The periplast is distinct and stains somewhat pinkish with Giemsa. The endoplasm is distinctly alveolar (Figs. 28, 39, 43), and concentration of protoplasmic granules occurs along the strands.

The undulating membrane (Figs. 34, 41—45) is very definite and stains particularly well with gentian violet and thionin. There are indications of myonemes (Figs. 41, 42, 44, 45) in some specimens, and this is not surprising, seeing that there are similar contractile structures in the membranes of Trypanosomes and of Spirochaetes.

The flagellum forms a chromatic border to the membrane. It appears thicker as the border of the membrane than it is as the free flagellum, for it gradually becomes more tenuous towards its free extremity (Figs. 33, 37, 46). The flagellum of *C. gerridis* does not originate from the blepharoplast. There is commonly a space between the two (Figs. 21, 25, 27) and one or more chromatic grains may be present in the intervening space. Sometimes one of these grains is so near the flagellum that the latter appears to arise from it (Figs. 22, 30, 32, 34, 43). Such a granule may be called a *basal granule.* No structure of the nature of a diplosome as described by Prowazek in *Herpetomonas muscae domesticae*, has been observed in this parasite.

The nucleus is usually oval (Pl. IV, Figs. 24, 25) or round (Figs. 35, 38). It may occupy the full breadth of the body of the parasite (Figs. 25, 31, 39) or not extend quite across (Figs. 21, 27, 32). Large chromatin granules (Figs. 34, 43, 45), occasionally eight in number (Fig. 31) are present. Usually the chromatin of the nucleus is arranged in slightly irregular masses (Fig. 27). Finer granules are found in the nuclei of parasites about to divide. Around the periphery of the nucleus is a chromatin condensation.

The blepharoplast. In addition to what has been previously stated, the following points with regard to the blepharoplast may be noted. Its chromatin stains very heavily with all the stains I used. The chromatin is much concentrated and usually the blepharoplast shows no sign of differentiation. The position of the blepharoplast is usually anterior to the nucleus (Pl. IV, Figs. 27, 39, 40) but very rarely it is laterally placed with regard to the nucleus. A clear area is not uncommon around the blepharoplast (Figs. 23, 44). The flagellum arises in the vicinity of the blepharoplast, often from a basal granule (Figs. 32, 43, 44).

Extra-nuclear chromatin—Chromidia. Chromidia are scattered at intervals in the general protoplasm of most of the *Crithidia* that I have examined (Figs. 26, 39, 44, 46). The chromidia seem more or less isolated in the alveolar protoplasm (Figs. 28, 39, 44). There is no indication of meta-chromatin being present such as Swellengrebel (1908) described for Trypanosomes.

The Post-flagellate Stage.

When the rectum of an infected *Gerris paludum* is examined, a certain number of flagellates are always to be found moving actively in its lumen, and rapid longitudinal division occurs, one parasite frequently giving rise to four smaller daughter forms. But one also finds that some of these small forms (*e.g.* Pl. IV, Fig. 36) have attached themselves to the wall of the gut or to portions of débris present in the gut. These parasites gradually cease their active movements, their bodies become less mobile and their protoplasm seems to concentrate towards the centre (Fig. 61). At the same time, the flagellum becomes shorter and is retracted nearer the body (Figs. 62, 63). The nucleus and blepharoplast approach one another (Figs. 63, 64), and if smears containing the parasite at this stage be stained, the major part of the rounded-up or oval body takes the chromatin colouration. The flagellum finally degenerates (Figs. 65, 66) and disappears (Figs. 67—69). It does not seem entirely to be absorbed, but would rather appear to be cast off by the parasite, for free flagella can be found in fresh preparations of rectal contents of *Gerris*. A thin, gelatinous layer now appears on the outside of the rounded-up organism and this layer rapidly shrinks and becomes hard, forming a closely adherent, fine cyst wall (Fig. 69). The *Crithidia* now consists of an oval body, containing nucleus and blepharoplast, and is very chromatoid in nature (Fig. 69). The cysts are 5μ to 6μ long by 3μ to 5μ broad. This post-flagellate (cyst) form is an obvious adaptation for life outside the host; it is expelled with the faeces of the bug and serves for the infection of a fresh host.

DIVISION.

Longitudinal division of *C. gerridis* may take place either towards the end of the pre-flagellate or during the flagellate stage as was pointed out by Patton (1908).

Longitudinal division as seen at the end of the pre-flagellate stage. The parasites about to divide become somewhat elongated and more or less oval in shape (Pl. IV, Fig. 16), and have a small flagellum. The blepharoplast first gives signs of division by elongating and becoming somewhat dumb-bell like (Fig. 17). It gradually constricts into two (Figs. 18, 19) and the halves separate. The flagellum splits simultaneously with the division of the blepharoplast, the division of the nucleus follows and the individuals show usually a bilaterally symmetrical form (Fig. 18). The general protoplasm becomes more tenuous at the long axis of the body and a split appears along it.

The two organisms so formed may separate at once or the division may be repeated, a rosette of parasites then resulting (Fig. 20).

As there is great variation in the size of the cysts ingested, there is also much variation in the size of the pre-flagellates exhibiting division.

Longitudinal division of mature flagellates. I have frequently watched the entire process of longitudinal division of the fully flagellated living forms. In many cases, the forms about to divide were slightly stouter than their neighbours, but this was not invariably so.

Division always seemed to be initiated by the blepharoplast becoming dumb-bell shaped and gradually dividing into two (Pl. IV, Fig. 48). This was followed by vigorous movements of the flagellum which began to split into two (Fig. 49) at the end near the blepharoplast. The nucleus also divided into two, the daughter nuclei migrating outwards to the periphery of the body (Fig. 49). The flagellum then executed rapid, lashing movements, and the split extended to the body (Figs. 50, 51, 52). The undulating membrane divided with the flagellum. After that, the progress of division was more gradual. The daughter organisms diverged, a Y being formed by their free portions with the undivided part (Figs. 53—56). Ultimately an angle of 180° was attained (Fig. 57), and the final separation occurred. Each daughter form thus produced had half of the nucleus, blepharoplast, membrane and flagellum of the parent. As a rule, division is equal (Figs. 54, 55) though, unlike Patton, I have sometimes seen markedly unequal division (Fig. 56). Possibly this is peculiar to the *Crithidia* of *G. paludum.*

By repeated longitudinal division rosettes of parasites are formed in the gut, but these very rapidly break up. In a true division rosette (Fig. 20), the flagella lie towards the periphery of the group, whereas in the more common aggregation rosettes (Fig. 47), the flagella serve as a centre of entanglement or are applied to the food particles to which the *Crithidia* cling, the posterior ends of the bodies of the parasites being outwardly directed.

Division of very narrow flagellates (Fig. 58) results in the formation of parasites that are almost spirochaetiform in appearance (Figs. 59, 60).

Polymorphism in C. gerridis, resulting from division.

The multiplication by division early in life and also at a more mature stage of existence is sufficient to account for the very great variation in size seen in the *Crithidia* of *Gerris paludum.* Some forms

(Pl. IV, Figs. 44, 45, 54) are enormously long—some 60μ to 90μ—compared with others. Small, short forms (Figs. 21, 29, 36, 48) also occur side by side with them. These small *Crithidia* have been produced by the division of young forms whose flagella were at the time but little developed. Stout parasites (Figs. 26, 37) are those as yet undivided. Repeated longitudinal division results in the production of very thin spirochaete-like individuals (Figs. 29, 59, 60) as before mentioned.

The great polymorphism exhibited by most insect flagellates is extremely well seen in *C. gerridis*.

At times it would be easy to mistake cases of sub-equal longitudinal division (Fig. 56) for conjugation of two flagellates, were the evidence of stained preparations only considered, or the division not watched from beginning to end in living specimens. This possibility of error has been pointed out by other workers on the group, and my own experience of *C. gerridis* and other *Crithidia*, and of *Herpetomonas*, leads me also to urge long and continuous observation of the living organisms as the only means of avoiding such errors.

Mode of Infection.

Gerris paludum, though a water-bug, very readily drowns if submerged. As evening approaches, the bugs have been noticed to seek the safer foothold afforded by flat-leaved water plants such as *Lemna major* and *Hydrocharis*, and rest, almost motionless, upon the surface of the leaves. When disturbed, blackish patches on the leaves due to faeces show where the *Gerris* have been. Examination of such faeces has shown that they contain post-flagellate "cysts" of *C. gerridis*. The *Gerris*, especially young nymphs, soon return to their former resting-places, and some at any rate, have been seen to suck up the moisture on the surface of the leaves, and thereby absorb the encysted *Crithidia*. This casual mode of infection has been proved for other species of insects.

The question of hereditary infection remains to be settled. Swingle (1908) has found developing forms of *Crithidia* in the ova of *Melophagus ovinus* infested by *Crithidia melophagia* and I have evidence confirming this observation. But in the case of *C. gerridis*, though parasites have been seen in the ovaries, no stage of development of the parasites has been seen as yet in the ova. These facts are not without biological significance, for they indicate a tendency towards the evolution of hereditary infection, which is actually attained in *C. melophagia*, where the *Crithidia* are found within the eggs and embryo of the *Melophagus*.

There is undoubted evidence that the *Gerris* becomes infected with *Crithidia* when the bugs are very young. Very small forms, the youngest nymphs obtainable, have been found to be parasitised, but this is explicable other than by hereditary infection.

The breeding grounds of the *Gerris* are quiet and sheltered spots, and defaecation follows oviposition very commonly. The young larva then is hatched in contact with faeces, some of which may be infected, and in its early quest of food, some of the crithidian cysts are doubtless ingested with the liquid taken up from the leaves, etc. around the young insect.

GENERAL REMARKS.

The genus *Crithidia* was founded by Léger (1902) for a flagellate that he found in *Anopheles maculipennis*. Two forms of the parasite were described by Léger; one consisted of a short, truncated body with a small flagellum, while the other possessed the usual long, tapering body, drawn out at its anterior end into the long flagellum, to which it was connected by a narrow, undulating membrane. From a study of the short, oval forms, Léger named the parasite *Crithidia fasciculata*, the name *Crithidia* denoting an oat. These short, oat-like forms would now be described as the late pre-flagellate stages of the parasite, and in this condition they are much like those of *Herpetomonas*. The study of the complete life-history of the parasites is necessary to determine fully to what genus any young form may belong.

Prowazek (1904) gave a life-history of a parasite, *Herpetomonas muscae domesticae* from the housefly, and stated that this organism had two flagella, a statement that has not been generally accepted. For some reason, the appearance of this memoir has caused much confusion of the genera *Herpetomonas* and *Crithidia*.

Lühe (1906) retains both genera, characterising *Crithidia* as possessing an undulating membrane and single flagellum, *Herpetomonas* as having no membrane but a long flagellum.

Novy, MacNeal and Torrey (1907) believe that Léger was dealing with a mixed infection of *Herpetomonas* and *Crithidia* in *Anopheles maculipennis*, which is quite possible; but these investigators characterise *Herpetomonas* as being the organism with the undulating membrane, their view therefore being quite opposed to that of all other workers on these organisms.

Patton (1908) described an insect parasite found in *Gerris fossarum, Microvelia* and *Perittopus sp.*, studied the life-history most carefully and divided it into three phases, a pre-flagellate, a flagellate and a post-flagellate phase. He followed out the entire development of the parasite, and showed the similarity between the pre-flagellate stages of a *Herpetomonas* and Léger's immature parasite, noting also the great likeness between the adult flagellate, *Crithidia fasciculata* and the adult *C. gerridis.*

Patton (1908, p. 142) sums up the situation thus:—"The genus *Crithidia* of Léger is therefore for the present best represented by all such flagellates in which the adult form is characterised by an attenuated anterior end to which the flagellum is attached by a rudimentary undulating membrane; the blepharoplast in all such flagellates is situated close up to the nucleus, never posterior to it."

Flu (1908) and Swingle (1908) working on a parasite recorded first by Pfeiffer (1905) from the gut of *Melophagus ovinus* have accepted the genus *Crithidia* as outlined above, and their flagellate is named *Crithidia melophagia.* I have worked myself on the latter parasite and consider it to belong to the genus *Crithidia.*

Patton (1909) has described a *Crithidia* from two species of Tabanids, and has written (XII. 1908) a critical review of the relations of blood sucking invertebrates to the life-cycle of the Trypanosomes of vertebrates. Dealing there with the vexed question of the three genera (*Trypanosoma, Crithidia, Herpetomonas*), he defines (p. 338) the genus *Crithidia* in the manner above stated, and in addition, the three characteristic stages in the life-cycle are mentioned—"pre-flagellate round or oval bodies with a nucleus and blepharoplast, which multiply by simple fission; flagellate stage, when they multiply by longitudinal division which may be either equal or unequal; in this stage they often exhibit marked polymorphism; post-flagellate stage, when the flagellates shorten, divide and then encyst, some species (in ticks, leeches, and *Melophagus ovinus*) pass this stage in the eggs of their hosts."

Having regard to the above definition, there is no doubt that the parasite with which I have been dealing is a true *Crithidia.* Further, Patton has described a similar organism from a very nearly allied host, another species of *Gerris.* Though the *Crithidia* here described from *Gerris paludum,* grows larger than that from *Gerris fossarum,* and has now been found in the ovaries as well as in the gut, the great similarities between the two *Crithidia* are such that it would be quite unjustifiable to create a new species for the parasite of *G. paludum.* It is probable that *Crithidia gerridis* is far more widely distributed among water-bugs than is at present known, and the present tendency to create new species of parasites, owing to *slight* differences of host, is to be deplored. Infection experiments are necessary to determine whether any parasite is absolutely restricted to one host or not, before exalting it to specific rank.

The exact systematic position of the genus *Crithidia* is somewhat difficult to determine. The possession of nucleus, blepharoplast and definite undulating membrane and flagella places it very near the Trypanosomes, but there is a difference in habitat, the majority of the Trypanosomes being blood inhabiting. Perhaps, however, this difference in habitat has been somewhat exaggerated, for the medium in which most of the *Crithidia* live is of much the same density as blood—in fact, *Crithidia melophagia* is commonly found mingled with either freshly drawn or partially digested blood of the sheep contained in the gut of *Melophagus ovinus.* Again, in the case of *Herpetomonas jaculum,* found in the gut of *Nepa cinerea,* the flagellate sometimes occurs in a medium containing fish's blood, when specimens of *Nepa* are examined just after attacking fish (sticklebacks). The importance of the density and composition of the medium need not be so great, then, as is generally supposed.

There is, then, little to debar *Crithidia* from a position in the family *Trypanosomatidae,* and when Léger created the genus *Crithidia,* he recognised the affinities of the two genera. The structure of *Crithidia* would place it somewhat higher in the scale of development than *Herpetomonas,* while its closer relation to the blood inhabiting Trypanosomes cannot be definitely stated until the early, probably pre-flagellate, stages of these latter parasites are known. The work of Moore and Breinl (1907) on the "latent" forms of Trypanosomes is very suggestive, as the latent forms are not unlike the post-flagellate stages of *Crithidia*[1].

SUMMARY.

(1) *Crithidia gerridis* occurs in the alimentary tract of *Gerris fossarum, Microvelia* and *Perittopus sp.* found in Madras. It is now

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[1] In this connection see the recent work of Bruel and his colleagues in Uganda on the development of *Trypanosoma gambiense* in the stomach of the tse-tse fly (*Proc. Roy. Soc.* Ser. B, Vol. 81, No. B. 550, published Oct. 27th, 1909). Pl. X, Figs. 15, 16 show obviously rounded up forms of Trypanosomes from the midgut of the fly, such forms apparently being capable of resistance for a long time.

recorded from the British water-bug, *Gerris paludum*, for the first time.

(2) The parasite occurs throughout the alimentary tract, in the ovaries and in the faeces of its host.

(3) There are three phases in the life-history of *Crithidia gerridis*, a pre-flagellate stage (Pl. IV, Figs. 1—10), a flagellate stage, and a post-flagellate stage (Figs. 64—69), the latter being adapted for life outside the body of the host, and for cross-infection.

(4) The adult flagellate has an elongate body, and possesses a large oval or round nucleus. There is also a smaller, usually rod-like mass of chromatin, the blepharoplast, near to which the flagellum arises. The flagellum may be as long again as the body, and is attached to it by a narrow, undulating membrane, in which myonemes are present (Figs. 22—46).

(5) The movements of the flagellated forms are characteristic; both the body and flagellum take part in the motion.

(6) The pre-flagellate forms are small, usually oval bodies, 3μ to 7μ long, and from 2μ to 4μ broad. Their nucleus often lies to one side and is round, the blepharoplast is rod-shaped. The flagellum grows out as a thin, delicate thread, in close contact with the body. The pre-flagellate phase of the parasite is found in the crop of the nymphs particularly, and also in the crop of the adults.

(7) The flagellate form has the general structure outlined in (4). The undulating membrane has indications of myonemes (Pl. IV, Figs. 41, 44, 45). The flagellum arises near a chromatic dot, the basal granule (Figs. 22, 30, 43). In the nucleus sometimes eight large chromatic masses may be present, or the grains may be very small. The nucleus on the whole is of the vesicular type. The blepharoplast is usually anterior to the nucleus, only very occasionally is it lateral. Chromidia are scattered in the endoplasm of the parasite (Figs. 26, 39, 46).

(8) The post-flagellate stage is a preparation for extra-corporeal life. The parasites divide in the rectum, lose their flagella, round themselves off and form a thin gelatinous cyst wall that rapidly hardens (Figs. 68, 69). These small cysts pass out with the faeces of the host.

(9) Longitudinal division is the chief method of multiplication. It may occur in all phases of the life-history and may be equal or subequal. The blepharoplast usually constricts first and division of it and of the flagellum follow one another very rapidly, division of the

membrane follows, and then that of the body. The daughter halves gradually diverge and finally separate. In pre-flagellate division, rosettes may be formed by several rapid, repeated, longitudinal divisions (Fig. 20). The fully flagellated individuals first divide into two and repeated division may result in rosettes, but these very rapidly break up. Aggregation rosettes of mature flagellates are, however, extremely common (Fig. 47).

(10) The mode of infection is a casual one, the young nymphs taking up faeces containing crithidian cysts from the leaves of water plants.

(11) The parasite is purely a parasite of insects, occurring in two species of *Gerris*, *G. fossarum* and *G. paludum*. The systematic position of the parasite is near the Trypanosomes in the family *Trypanosomatidae*.

(12) There is much variation of form exhibited by the adult flagellate. Some of the very long parasites (Pl. IV, Figs. 39, 44—46). appear to be peculiar to the *Crithidia* found in the gut of *G. paludum*, and have not been figured before so far as I know. This polymorphism needs careful attention, and is very confusing if only isolated stages of the parasite are studied. A similar remark applies to other crithidial and herpetomonad forms.

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EXPLANATION OF PLATE IV.

All figures were outlined with an Abbé-Zeiss camera lucida, using a 2 mm. apochromatic or $\frac{1}{12}$ inch achromatic (Zeiss) objective and compensating ocular 8 of Zeiss.

The magnification is, in all cases, approximately 1300 diameters.

Fig. 1. Pre-flagellate, recently ingested. Nucleus shown to one side, blepharoplast distinct. Crop of *Gerris* Nymph. Delafield.

Figs. 2—4. Larger pre-flagellates than in 1. Crop preparation. Giemsa.

Fig. 5. Pre-flagellates with blepharoplast in contact with nucleus. Crop of adult. Giemsa.

Fig. 6. Pre-flagellate showing very early development of flagellum from the neighbourhood of the blepharoplast. Flagellum lying close to the body. Crop of adult. Gentian violet.

Fig. 7. Parasite with longer flagellum. Crop of adult. Giemsa.

Figs. 8—10. Parasites showing still longer flagella coiled on the body but not yet free from it. Crop of adult. Giemsa.

Fig. 11. Small form with flagellum free from the body. Crop of adult. Giemsa.

Fig. 12. Oval pre-flagellate showing the commencement of the elongation of the posterior end. Crop of nymph. Giemsa.

Fig. 13. Very rounded and large form of pre-flagellate, with round blepharoplast. Crop of adult. Delafield.

Figs. 14, 15. Late pre-flagellates showing progressive elongation of the posterior end. Crop of adult. Giemsa.

Figs. 16—19. Stages in the division of pre-flagellates. Crop of nymphs.

Fig. 16. Undivided form of parasite. Large specimen. Nucleus round, blepharoplast entire. Flagellum coiled on body. Thionin.

Fig. 17. Young dividing form. Blepharoplast almost constricted in two; nucleus bilobed; flagellum split at the body end.

Fig. 18. Division almost completed. The organism shows two blepharoplasts, two nuclei, two flagella, and a clearer area marks the line along which separation of the bodies of the daughter forms occurs.

Fig. 19. Division of pre-flagellate as seen at a different plane from that shown in Fig. 17. Crop of nymph. Delafield.

Fig. 20. Multiple longitudinal division of small pre-flagellates showing resultant rosette formation. Crop of nymph. Gentian violet.

Fig. 21. Small flagellate. Nucleus and blepharoplast well shown. Stomach of adult. Gentian violet.

Fig. 22. Larger flagellate. Blepharoplast slightly oblique, extending right across body. Intestine of adult. Giemsa.

Fig. 23. Form with somewhat slender posterior end and "peg-top" like appearance. Crop. Delafield.

Figs. 24, 25. Forms showing vesicular nuclei and distinct flagella. Stomach. Giemsa.

Fig. 26. Flagellate with long drawn out body. Alveolar protoplasm, containing chromidia. Rather compact nucleus. Stomach. Gentian violet.

Fig. 27. Crithidia with very well-marked flagellum. Stomach. Gentian violet.

Fig. 28. Flagellate with well-marked chromidia and alveolar protoplasm. Stomach. Thionin.

Fig. 29. Narrow form of flagellate. Intestine. Giemsa.

Fig. 30. Flagellate showing twist on the body. Crop. Delafield.

Fig. 31. Flagellate with protoplasm perhaps suggestive of a cytopharynx. Stomach. Thionin.

Fig. 32. Flagellate with large chromatic basal granule near the blepharoplast from which the flagellum arises. Stomach. Thionin.

Fig. 33. Narrow form with flagellum showing only as a line along the body. Intestine. Giemsa.

Fig. 34. Parasite showing rather wide membrane. Nucleus with regularly arranged chromatin granules. Vacuole at posterior end. Intestine. Giemsa.

Fig. 35. Flagellate with well-marked round nucleus and myonemes in membrane. Intestine. Delafield.

Fig. 36. Short form common in the rectum. Delafield.

Fig. 37. Rather broad flagellate with well-marked flagellum. Stomach. Thionin.

Fig. 38. Flagellate showing "peg-top" form and large, round nucleus. Intestine. Delafield.

Fig. 39. Very long form with well-marked oval nucleus, alveolar protoplasm and chromidia. Very pointed posterior end. Stomach. Gentian violet.

Fig. 40. *Crithidia* showing broad flagellar end. Intestine. Gentian violet.

Fig. 41. Short parasite showing myonemes in the membrane. Crop. Delafield.

Fig. 42. Longer form with well-marked myonemes in the membrane. Chromidia near the anterior end. Stomach. Delafield.

Fig. 43. Long parasite with broad anterior end, alveolar protoplasm and large basal granule. Intestine. Gentian violet.

Fig. 44. Large form of *Crithidia* with very well-marked thick flagellum and myonemes in membrane. Rather broad posterior end. Crop. Delafield.

Fig. 45. Extremely long *Crithidia* with well-marked nucleus and blepharoplast; membrane and myonemes very distinct. Stomach. Gentian violet.

Fig. 46. Narrow parasite with very marked flagellum. Intestine. Delafield.

Fig. 47. Portion of aggregation-rosette of *Crithidia gerridis* massed around a food particle and parasites attached to the particle by their flagella. Stomach. Thionin.

Figs. 48—58. Stages in division.

Fig. 48. Early stage of division of flagellate. The blepharoplast has divided, the flagellum has begun to divide. Intestine. Thionin.

Fig. 49. Further stage in division. Two flagella, nuclei and blepharoplasts are present. Stomach. Giemsa.

Fig. 50. Dividing form showing cleft appearing between the bodies of the daughter forms. Stomach. Thionin.

Fig. 51. Form showing division of membrane. Stomach. Thionin.

Fig. 52. Further stage in division. Stomach. Delafield.

Fig. 53. Two almost separated daughter *Crithidia*. Stomach. Thionin.

Fig. 54. Very long form dividing. The organism has divided at the anterior end while the posterior end of the body is twisted. Intestine. Delafield.

Fig. 55. Almost completed equal longitudinal division. Daughter parasites forming a Y. Rectum. Giemsa.

Fig. 56. Marked sub-equal division. The lower daughter organism is much broader than the upper one. Stomach. Gentian violet.

Fig. 57. Daughter organisms about to finally separate, placed at an angle of 180° with one another. Stomach. Thionin.

Fig. 58. Division of very narrow form giving rise to almost Spirochaetiform *Crithidia*. Rectum. Thionin.

Fig. 59. Very narrow, almost Spirochaetiform *Crithidia*. Rectum. Thionin.

Fig. 60. Spirochaetiform *Crithidia* showing "peg-top" like arrangement. Rectum. Thionin.

Figs. 61—69. Post-flagellate stages, from rectum and faeces.

Fig. 61. Commencement of encystment. Posterior end withdrawn. Gentian violet.

Fig. 62. Parasite rounding up. Anterior end less distinct. Giemsa.

Fig. 63. Anterior end almost withdrawn. Flagellum disappearing. Giemsa.

Fig. 64. Pear-shaped organism. Flagellum almost gone. Giemsa.

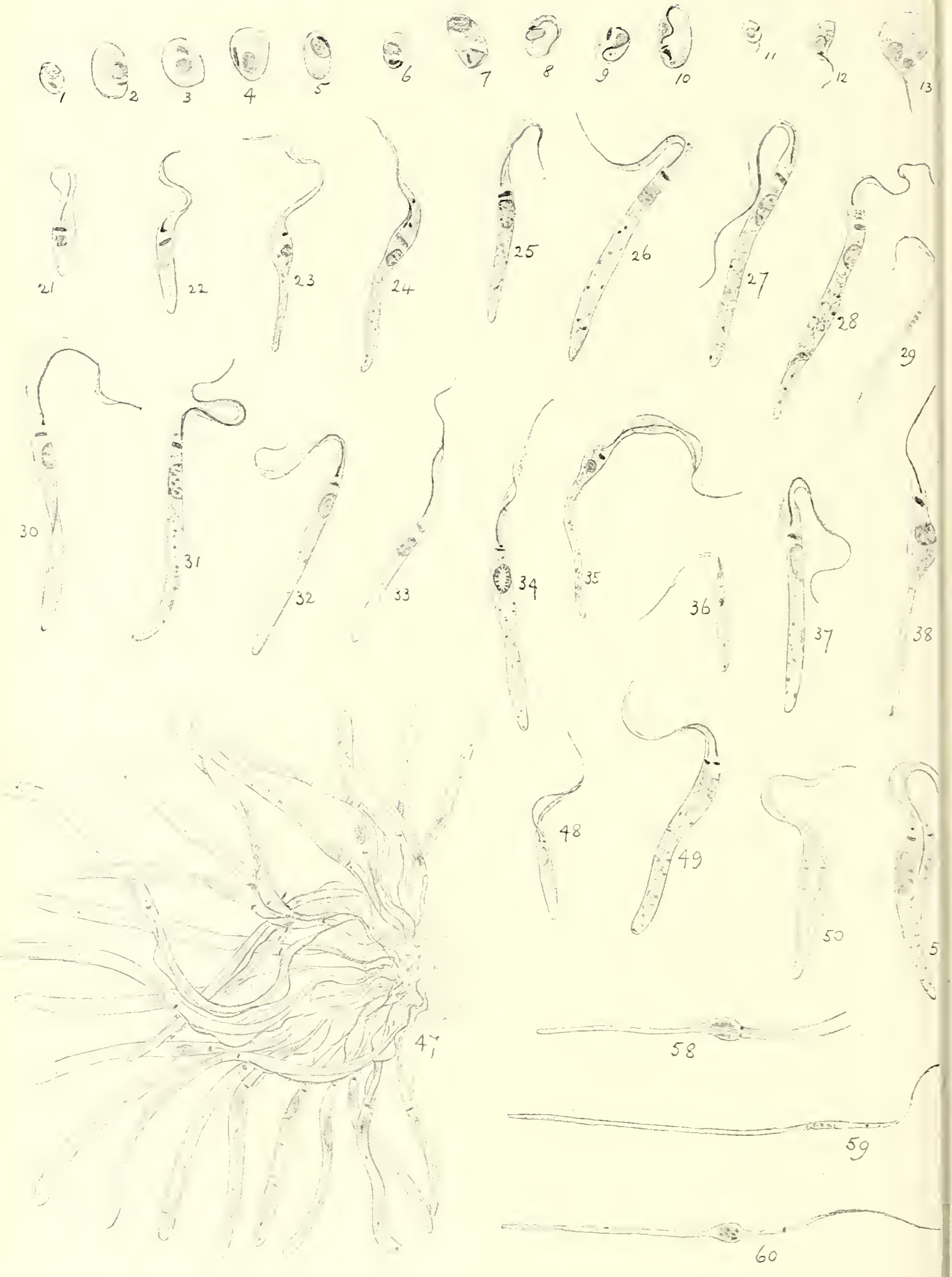
Fig. 65. Small, rounded form flagellum forming a tag. Thionin.

Fig. 66. Larger form than in Fig. 65. Very distinct blepharoplast. Gentian Violet.

Fig. 67. Flagellate almost encysted. Delafield.

Fig. 68. Cyst with round nucleus and distinct blepharoplast. Delafield.

Fig. 69. Encysted *Crithidia* with blepharoplast in contact with the very large nucleus. Delafield.



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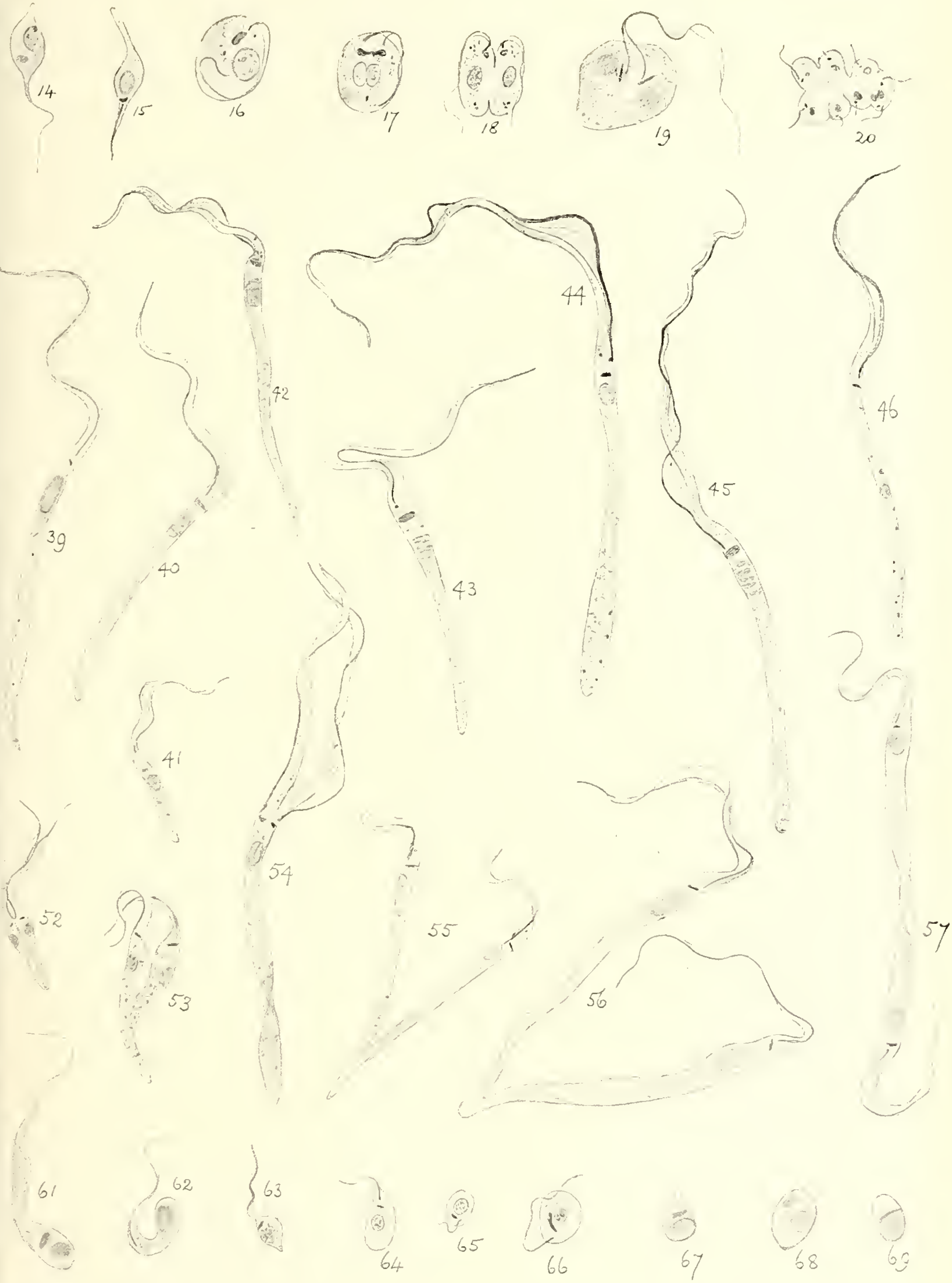
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HISTORY

THE LIFE-CYCLE OF *HERPETOMONAS JACULUM* (LÉGER), PARASITIC IN THE ALIMENTARY TRACT OF *NEPA CINEREA*.

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(With Plate V and 1 Text-figure.)

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Introduction.

Among the flagellates to which much attention has been directed recently, the genera *Trypanosoma*, *Crithidia* and *Herpetomonas* are prominent, on account not only of their intrinsic interest, but because of the connexion of various members of these genera with well-known diseases, some of which may affect man. The genus *Herpetomonas*, with a species of which this paper is chiefly concerned, has one of its members connected with the disease known as Kala-azar in India, while the parasites of infantile splenomegaly and of Oriental sore are also developmental stages of Herpetomonads. In the case of Kala-azar, the adult flagellates have been seen to develop in the bed-bug, *Cimex rotundatus.*

The subject of this paper is, however, not a pathogenic agent, so far as I am aware, but lives, apparently harmlessly, in the alimentary tract of the water-scorpion, *Nepa cinerea.* The flagellate stage of the parasite was first described by Léger in 1902, and was named by him *Herpetomonas jaculum.* In this paper I hope to give, for the first time, the *complete* life-history of the parasite.

Material and Methods.

The "common" water-scorpion does not appear to be so common nowadays as its name would imply. Though many likely localities in the south-east of England have been tried, yet the majority failed to yield specimens of *Nepa cinerea.* However, many adults and a fairly large number of nymphs were obtained. Most of the *Nepa* were infected with the *Herpetomonas.* Specimens have been examined at all seasons of the year. It is only in the nymphs of *Nepa* that the pre-flagellate stages can be examined with any degree of ease.

Each *Nepa* was carefully dissected[1] and the intestine removed. This was divided into portions which were teased in physiological salt solution, and the parasites were fixed wet either by osmic vapour or the vapour of strong formalin, both of which were efficient, the osmic fixation being perhaps slightly the better. The preparations were then spread, without being allowed to dry. Preparations of the ovaries were made in the same way. Cover-glass smears were sometimes made. Most of the smears were treated throughout by the wet method, while others were allowed to dry before being stained. Other preparations were made in which Bouin's liquid and corrosive-acetic-alcohol were used as fixing agents.

For observations *in vivo* hanging-drop preparations were used, or else preparations of the infected gut in a little physiological salt solution were placed under a cover-slip, the edges of which were sealed with vaseline. The parasites could then be watched for a considerable time. In very young specimens of *Nepa* the gut was extremely transparent, and the parasites could be seen moving about within it.

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[1] During my investigations I have seen various stages, especially spores, of the Coccidium, *Barroussia ornata* (A. Schneider), in the gut-epithelium and Malpighian tubules of *Nepa cinerea.*

For purposes of comparison several stains were used. Giemsa's stain was of great service in giving a general idea of the morphology of the parasite, though the tendency of this stain to exaggerate the chromatin needs to be checked by the use of others. Iron-haematoxylin followed by orange-G, thionin, and Delafield's haematoxylin, either alone or followed by orange-G, were all found of much service. Twort's stain (neutral-red and Lichtgrün) and safranin were also tried.

Intra-vitam staining was not of much assistance, but methylene-blue proved superior to neutral-red.

General Description.

Herpetomonas jaculum has three phases in its life-history. Taking as the first, the pre-flagellate stage, the parasite exists as a small, oval, cyst-like body (Pl. V, Figs. 1—6), possessing two chromatin masses, that is a true nucleus and a blepharoplast. The flagellum arises near the blepharoplast, elongates and grows out, till the organism gradually reaches the second phase of its life-history, that of the fully formed flagellate.

The flagellate form (Pl. V, Figs. 19—36) is the most commonly occurring one. The body of the flagellate parasite is from 13 μ to 33 μ long and from 1 μ to 4 μ broad. The free flagellum is about as long again as the body. The body is elongate and is rather blunt at its flagellar end, not so tapering as is the case in *Crithidia.* The long, gregariniform, oval body shows clear ectoplasm and a granular endoplasm, though granules are far less numerous than in *Crithidia,* nor is the endoplasm so alveolar in nature. Each parasite possesses an oval nucleus, usually nearer the flagellar end of the body, and a rod-shaped blepharoplast is present, near to which the flagellum arises. A single flagellum is present, but in many adult flagellates there is an appearance of two flagella near the body end (Figs. 38, 39), as the flagellum divides precociously, even before the organism has completed its growth, and before the body as a whole divides longitudinally. The process of division takes place repeatedly during the flagellate stage of existence. After a number of successive longitudinal divisions, some of the forms resulting therefrom (Figs. 51, 52) reach the rectum. Here again division longitudinally occurs and the result is the production usually of four small flagellates from one. These forms gradually lose their flagella, become rounded off and are passed out along with the faeces of the *Nepa*, as small, post-flagellate cysts which are specially adapted for resisting desiccation and serve to infect new hosts (Figs. 57—68).

Movements.

Herpetomonas jaculum possesses a single flagellum and this is very noticeable when the organism is moving. The adult flagellates are either fixed by their flagellar ends to the gut wall of their host or else they swim actively in the gut contents. The attached forms are usually in clusters or aggregation rosettes (Fig. 45), and their movement is suggestive of that of ciliated epithelium. The body of each flagellate bends slightly and rhythmically sways to and fro, and at the same time it twists on itself, so that what was at first the upper surface becomes the under one, and the previously under one becomes exposed to view. (Cf. Fig. 23.) There is no definite direction for this twist ; I have seen forms twist from right to left, then reverse their motion and twist from left to right. This rolling movement of *Herpetomonas* has been mentioned by other writers (Léger, Patton, Berliner) but has not been described in detail.

The movements of free flagellates may next be considered. These are far more rigid and jerky in character than those of *Crithidia*. This is to be expected, seeing that they possess no undulating membrane, the presence of which tends to produce smoothness of motion. The same rolling movements as are seen in attached flagellates, can be observed in free forms. But in addition there are the rapid lashing movements of the flagellum. The flagellum lashes from side to side and frequently is thrown into coils, so that it bores its way spirally through the medium in which the flagellate swims, and much focussing is necessary to observe the full length of the flagellum. As a rule, the flagellar end of the organism moves in the forward direction, the flagellum then functioning as a tractellum, but on occasions, I have seen the *Herpetomonas* moving fairly rapidly, its flagellum trailing as a feeble pulsellum and its blunt, posterior end moving forwards. The rapidity of progression under these circumstances is not so great as when the anterior (flagellar) end is forwardly directed.

A further aid to movement forwards is afforded by the contractions of the body itself, for myonemes are present. Waves of contraction pass along the body-myonemes (Pl. V, Figs. 20, 21) from the posterior end towards the flagellum. The appearance of the organism is entirely altered thereby, the waves causing the flagellar end to swell out and become ball-like, so that the organism more or less resembles a peg-top. Relaxation follows this contraction, and the flagellar end seems to spring forwards with a jerk, this really being due to the release of the tension of the ectoplasm of the rounded part of the organism. The rolling movements continue concurrently with those of contraction. As I have seen this movement occur while the *Herpetomonas* was within the unruptured gut of the *Nepa*, I do not think it can be regarded as an abnormal one.

Reversal of the direction of movement frequently occurs. It is accomplished very rapidly. The flagellum swings out suddenly in a semi-circular direction, and so great is the impetus of movement, that the body is swung round by the flagellum, and the two come to lie in a straight line with one another. The spirally boring and lashing movements of the flagellum, usually accompanied by those of contraction and relaxation of the body, are re-commenced, and the organism moves away in the direction opposite to that in which it was progressing before.

Sometimes a parasite is seen whose flagellum hardly moves at all and yet it progresses forwards with ease. A gentle, undulating movement of the body from side to side is all that is obvious, the movements of the flagellum being so slight as to be hardly noticeable.

Many peculiar movements, due to the flagella of two parasites becoming entangled, have been seen. Organisms entangled thus have rotated for many minutes with great speed, the point of entanglement acting as a centre about which the rotation occurred. These forms have invariably separated from one another finally and swam away.

Morphology.

There is much variation in the gross morphology of *Herpetomonas jaculum* as seen in different parts of the gut of the host. Small rounded or oval pre-flagellates and later pre-flagellates are most abundant in the crop; fully flagellated organisms are specially abundant in the stomach and early part of the intestine, while in the rectal portion of the intestine there are numerous short forms, which are in process of division and also of encystment. These post-flagellates also occur in the faeces.

Flagellates occurring in male *Nepa cinerea* are often much more slender than those obtained from females. Parasites occur in the ovaries and are very broad. They have not been found in the testes of *Nepa*. The significance of the forms in the ovaries will be discussed later.

The Pre-flagellate Stage.

The pre-flagellate stage of *H. jaculum* is best observed in the crop of the young nymphs of *Nepa cinerea*. Here may be found, together with some fully formed flagellates, all forms leading up to the adult flagellate. Commencing as small cysts (Pl. V, Fig. 1), probably ingested with the faeces of another *Nepa*, the adult flagellate is gradually developed. The oval pre-flagellates (Figs. 1—6) measure from 4μ to 5μ long, and from 2μ to $2{\cdot}5\mu$ broad.

Each young form becomes somewhat oval. Internally it consists of a nucleus, usually oval (Pl. V, Figs. 1, 2) or round (Fig. 12), and lying often to one side of this is a more or less rod-shaped blepharoplast (Figs. 1, 4, 15), surrounded by the cytoplasm. In this respect *H. jaculum* is very like *H. lygaei* as described by Patton (1908). An area (Figs. 2, 4) staining much like chromatin and somewhat variable in position is often present. As growth proceeds, a thread, staining pink with Giemsa and purple with Delafield, makes its appearance at the side of the organism (Figs. 5, 6), having originated from the area before mentioned, and near to the blepharoplast. This thread grows out as a kind of small tag at first near the body (Figs. 7—10), later extending beyond it (Figs. 11—14) and sometimes gradually drawing out the body after it for a very short distance only (Figs. 15, 16). The end of the body opposite to the flagellum also elongates (Fig. 17), the body becomes spindle-shaped and the appearance of the adult flagellate then is assumed.

The Flagellate Stage.

The following points regarding this, the best known stage of *Herpetomonas jaculum*, are in addition to those given in the general description.

The ectoplasm is clear and flexible. Myonemes (Pl. V, Figs. 20, 21, 38) are present in it and can be seen on staining somewhat deeply. The contractile movements of the body are in part due to these organellae.

The endoplasm as before stated is fairly granular. At times in living specimens, the granules are so arranged as to suggest the presence of a cytopharynx between them. But further observation of the living creature showed the obliteration of this feature after a short time, and I was very rarely able to find it in fixed and stained preparations (Pl. V, Fig. 22). Léger (1902) has described such an "intestinal canal" in the *Herpetomonas* of the *Nepa* he examined, but I have never been able to establish definitely its existence in the parasites of the British *Nepa*. But on several occasions I have seen a markedly clear area (Figs. 25, 28) near the posterior end of the parasite, such a vacuole (function undetermined) having been described by Léger.

The nucleus[1] is usually oval in form, its long diameter coinciding with that of the body. In the early flagellate stages, the nucleus is fairly compact, but as growth proceeds, it becomes more vesicular (Pl. V, Figs. 21, 22, 25) and its chromatin takes the form of small grains scattered through the general nucleoplasm. In some fully developed flagellates about eight masses of chromatin are present (Figs. 28, 31, 34, 36). I have not been able to establish any definite number of chromosomes for the nucleus of *H. jaculum* as Patton (1908) was able to do for *H. lygaei*, but I agree with Léger that there are a number of chromatin grains present. Possibly this is due to the fact that division of *H. jaculum* is initiated extremely early, and the finely divided condition of the chromatin is one well-suited for equal distribution of chromatin between the daughter forms resulting from division.

The blepharoplast (kinetonucleus) is comparatively large and stains very deeply. A clear area is often seen around it (Pl. V, Figs. 16, 18, 28). It is usually rod-shaped (Figs. 19, 22, 28), sometimes slightly curved (Figs. 30, 31). It may be placed at right angles to the long axis of the body (Figs. 22, 25) or may be inclined at an angle to it (Figs. 19, 32). On one occasion I found several specimens of *H. jaculum* with the blepharoplast lying parallel to the long axis of the body (Fig. 24), but this is most uncommon. The blepharoplast is almost invariably situated in the pre-nuclear region of the body of the parasite. In division the blepharoplast becomes dumb-bell shaped and constricts into two (Figs. 37, 38), while in encystment it approaches the nucleus. I have frequently seen constriction of the blepharoplast into two, but

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[1] Throughout this paper the terms *nucleus* and *blepharoplast* have been used to denote the two chief chromatin bodies of the parasite, for these terms have been used consistently by practically all workers on *Herpetomonas* and *Crithidia* and thus have received international recognition.

I have never been able to find any evidence of complicated reducing divisions such as Prowazek (1904) described in the case of *Herpetomonas muscae domesticae.*

Chromidia are found in *H. jaculum* and are usually widely separated one from another (Pl. V, Figs. 28, 33, 34). They are not so abundant as in *H. lygaei.* Chromidia occurring either singly or in small groups near the posterior end of the body of *H. jaculum* probably explain the "posterior diplosomes" of some authors (Figs. 28, 32—34, 37).

The Flagellum is very noticeable in *H. jaculum.* It develops in the late pre-flagellate stage of the parasite from a chromatin-like staining area near the blepharoplast. The flagellum emerges from the body as a *single* element (Pl. V, Figs. 11—16), and remains single for a short time, whilst the body of the *Herpetomonas* elongates (Figs. 15, 16, 17) and gradually assumes the typical, gregariniform appearance (Figs. 19—36). The flagellum is much thicker at its origin than it is distally. A basal granule (blepharoplast of Minchin) (Figs. 8, 12, 22, 34, 35) is very near the origin of the flagellum, at times touching it, and lies between the root of the flagellum and the blepharoplast.

The organism at this stage is ready for active multiplication by division, and slight constriction of the blepharoplast appears, accompanied by a separation of the lower part of the flagellum into two portions (Pl. V, Figs. 37, 38). The condition of the organism at this time resembles that figured by Prowazek (1904) for *H. muscae domesticae.* Now, it frequently happens that practically every *Herpetomonas jaculum* in one *Nepa* is in this condition at the same time, and as a lull in the process of division occurs here, it would be very easy to mistake this condition for that normally present in the parasite, and to state, as Prowazek has done for *H. muscae domesticae,* that two flagella united by a membrane are present.

Having recently examined *Musca domestica* for the purpose of comparing *H. muscae domesticae* directly with *H. jaculum,* I found that in many flies, every *Herpetomonas* in each fly showed but a single flagellum; also that the flagellum commenced to divide before the rest of the organism, but that the split never extended very far—in fact, that the division was delayed for some time after its initiation. In other flies, practically every *H. muscae domesticae* seen exhibited the so-called "double flagellum," and in the former cases, the phenomenon is only that of precocious division of the flagellum. The matter can only be finally decided in *H. muscae domesticae* by carefully tracing out the development of the flagellum from the cyst or post-flagellate form—living material, and not stained preparations, being the basis of observations. In *H. jaculum* the condition of the organism with the single flagellum is the normal one, and the double flagellum (Pl. V, Figs. 37, 38) is to be associated with division, as it is in the case of the *Crithidia*, Trypanosomes and Spirochaetes. I have never seen the origin of the "second flagellum" by a process of sprouting, such as Berliner[1] (1909) describes.

Though I have very carefully searched for rhizoplasts and diplosomes such as Berliner figured in *H. jaculum*, I have never found a single specimen showing this feature, no matter what stain and what magnification was used.

The flagellum always was continuous and tapered gradually from its origin near the basal granule to its free extremity (Pl. V, Figs. 34, 36). A very slight thickening is occasionally present (Fig. 28) where the flagellum leaves the body, but this is the only indication of an anterior "diplosome" that I have ever seen, and it is my firm conviction that this structure as well as a rhizoplast is absent from the specimens of *H. jaculum* that I have examined.

Regarding the exact nature of the flagellum, from a consideration of all its staining reactions, it would seem to consist largely of chromatin or a substance very closely allied thereto. If a cytoplasmic stain be used strongly (*e.g.* orange-G or Lichtgrün) an extremely tenuous cytoplasmic layer is seen around the chromatoid core. It is probable, then, that the flagellum of *H. jaculum* consists of a thin thread of chromatin with a film of cytoplasm around it.

The Post-flagellate Stage.

This phase of the parasite of *H. jaculum* is destined for the infection of new hosts. Preceding the formation of the actual post-flagellate or cyst, the *Herpetomonas* often attach themselves to the wall of the gut or to débris. A doubly repeated longitudinal division of the adult parasite (Pl. V, Figs. 51—55) then occurs, during which the flagellum gradually disappears. The resulting individuals (Fig. 56) are very small, and are practically motionless. They consist of a nucleus and blepharoplast, together with a small amount of general cytoplasm. They gradually concentrate their bulk, a thin, gelatinous cyst wall that rapidly hardens is secreted around each, and they become small, oval bodies, that stain very deeply on account of the large amount of chromatin that they contain (Figs. 57—68). The post-flagellate cysts measure from 2·5μ to 4·5μ long and 1·4μ to 2·6μ broad.

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[1] My researches on *H. jaculum* were completed before Berliner's account was published.

The tiny, oval, very resistant cysts become detached from the rectal walls, and pass out with the insect's faeces, that form minute, blackish patches on the leaves of water plants.

A form of the post-flagellate stage occurs in the ovaries of the host. Thus very large, broad, non-flagellated parasites (Pl. V, Figs. 46—49) were found in the ovaries of most of the female *Nepa cinerea* that I examined. These forms have a well-marked nucleus and blepharoplast (Figs. 47, 48, 49). Their protoplasm is very alveolar (Figs. 46, 49) and chromidia (Figs. 46—49) are much more numerous than in parasites inhabiting the gut. I have seen *H. jaculum* pass through the walls of the gut, and swim towards and penetrate the ovaries. The parasites appear then to lose their flagella, and to assume the form described above. Since careful examination of both ova and embryos has never shown the presence of *H. jaculum*, it is likely that these very large parasites simply undergo degeneration in the ovaries; the abundance of chromidia in the general protoplasm tends to confirm this supposition. Nevertheless, the occurrence of *H. jaculum* in the ovaries is of interest, representing, as it does, an attempt at the evolution of hereditary infection. It is of interest to note that Prowazek (1904) found *H. muscae domesticae* in the ovaries of *Musca domestica*.

Division.

Longitudinal division is the means of rapid multiplication of *H. jaculum*. It occurs either in the pre-flagellate stage, whereby the present host is primarily infected (Pl. V, Figs. 2, 3, 4), or in the flagellate stage (Figs. 37—44), or in the post-flagellate phase (Figs. 51—54). In the case of post-flagellates very rapid longitudinal division of a single organism gives rise to several—usually four—small daughter forms that proceed to encyst.

The division of the *pre-flagellate* forms begins with the division of the blepharoplast. This becomes dumb-bell shaped and gradually constricts into two (Pl. V, Fig. 2), the daughter halves migrating frequently to the opposite sides of the organism. Vacuoles appear in the general protoplasm, and gradually fuse in the mid-line, so that in the living specimens a clear area is seen posterior to the nucleus (Figs. 2—4). Most of the chromatin of the nucleus, meanwhile, has become very finely granular, very few large granules remaining. The nucleus in turn gradually constricts in two, the line of separation coinciding with that of the long axis of the body of the organism (Figs. 2, 3, 4). Separation ultimately occurs, the ends nearer the blepharoplasts first becoming free.

Division is sometimes repeated before the daughter organisms separate, and a rosette of parasites is thus formed (Pl. V, Fig. 18). The members of a rosette do not acquire their flagella simultaneously, and they separate when the flagella are formed; consequently groups containing both flagellated and non-flagellated forms are found (Fig. 18).

The division of the *flagellated* forms usually commences with that of the flagellum, though the blepharoplast in such cases constricts at the same time (Pl. V, Figs. 37, 38). Further division of the flagellum may be arrested considerably. Longitudinal fission proceeds as in the pre-flagellate forms. The blepharoplast and nucleus divide (Figs. 39, 40), and the daughter nuclei so formed migrate to opposite sides of the organism. The flagellum then divides (Fig. 39) and separation of the bodies commences (Figs. 40, 41, 42). The separation is aided by the vigorous movements of the flagella, which cause a very rapid divergence of the daughter Herpetomonads (Fig. 43) until they are in a straight line with one another (Fig. 44) when final separation occurs. Repeated longitudinal division results in the production of very narrow, slender forms (Fig. 35).

Post-flagellate division is really of the nature of a preparation for encystment. When about to divide the parasite shortens and thickens. Division of the ordinary flagellate type then occurs (Pl. V, Fig. 51). As division proceeds (Fig. 52) the body shortens, the protoplasm concentrates around the nucleus, and the flagellum appears either to be partly absorbed or to degenerate (Figs. 52, 53), for it gradually disappears (Figs. 53, 54). A second division then occurs (Figs. 54, 55); the tiny oval daughter forms (Fig. 56) show no flagellum, and gradually, as a gelatinous cyst is secreted around them, form small cysts (Figs. 57—68).

Sometimes the two daughter forms and the cysts they produce remain side by side (Pl. V, Fig. 62). At other times they separate and become isolated. Groups of cysts (Fig. 68) are not uncommon in the rectum. During the division prior to encystment, the flagellates may fix themselves to the walls of the rectum, and consequently the cysts are attached to some extent to the gut wall, but not so firmly as to prevent them being detached and passing out with the faeces of the host.

Sexuality in Herpetomonas jaculum.

Much has been written from time to time of sexual dimorphism in the *Trypanosomatidae.* I do not propose to enter into the subject very fully here, except in so far as it relates to *Herpetomonas.* Up to the present Prowazek appears to be the only writer who has attributed sexual characters to *Herpetomonas.* According to Prowazek *H. muscae domesticae* has three forms of individuals, male, female and indifferent, size and granulation being the criteria for their separation. Apparently the involved processes described by Prowazek as occurring before, during and after copulation, were only studied by him in stained preparations. Copulation has apparently never been observed *in vivo* in the *Trypanosomatidae.*

Size as a criterion of sex does not hold, for the following reasons:

(*a*) *Herpetomonas jaculum* obtained from large, adult *Nepa* are frequently larger than those from the nymphs.

(*b*) *H. jaculum* in the rectum divides very rapidly, and I have watched one individual divide and produce two daughter forms, each of which again divided. But these very small forms were not male forms, for they lost their flagella and became encysted—but if thinness and small size were to be considered, emphatically they should have been male forms and should have behaved accordingly.

(*c*) Regarding the presence of much granular protoplasm as being characteristic of female forms and its absence being the usual condition in males, I would cite the following experiments:

On one day I collected a number of active, healthy *Nepa,* and dissected half of them, the others being put into an aquarium. All of those dissected had recently fed on blood, either of a fish or of an amphibian, unchanged nucleated red corpuscles being present in the blood contained in the crops of the *Nepa.* All were infected with *H. jaculum,* and in every case the body of the parasite contained very many large, refractile granules, apparently of reserve food material. The bodies of the organisms also were broad. My preparations, if the sex criteria quoted above be called into consideration, contained female Herpetomonads. Three days later, I dissected the remaining *Nepa,* that had not been fed during that period. They, too, were infected with *H. jaculum,* but the Herpetomonads were long, slender forms, whose bodies showed few or no granules in them: in other words, they were "male" forms. I interpreted the occurrence of few granules in these forms as the result of the Herpetomonads being deprived of food, and tried the following experiments which confirm this view.

A *Nepa*, fed on larvae of *Chironomus*, was dissected after a few hours and the fluid removed from its crop by slight pressure. No Herpetomonads were found in this liquid, though naturally I made a very careful search. Some of the crop fluid was added to a preparation of *Herpetomonas jaculum* from starved *Nepa*, and the effect of it on the parasites was noted. The Herpetomonads became visibly more granular. I consider, therefore, that the presence of granular protoplasm is not a criterion of the female sex but is merely a manifestation of great metabolic activity. Although some biologists would perhaps hold that these granular forms were potential females, I never saw copulation, even when these very granular thick forms and thin, non-granular forms were intermingled in the same medium.

Occasionally I have caught sticklebacks (*Gasterosteus aculeatus*) with *Nepa* attached to them, their proboscides buried deep in the fish. As these *Nepa* were infected with *H. jaculum*, the parasite occurred naturally mixed with vertebrate blood in the gut contents of *Nepa*. The Herpetomonads also were richly granular forms. Fresh human blood from a pricked finger was added to *H. jaculum* from starved *Nepa*. The protoplasm of the parasites became more granular, their movements were more active and longitudinal division occurred. The parasites lived for several hours. The granules were those of reserve food material as judged by their deep-staining reactions (*intra-vitam*) with methylene blue. The *Herpetomonas* certainly showed no signs of degeneration, and this experiment, I believe, conclusively shows that the occurrence of many granules in a parasite is to be associated with metabolism, not with sex.

(*d*) By measuring many parasites, a series of forms may be obtained —from small to large—whose breadth and length gradually increase. Where, in a series, is the limit say for male forms to be applied? It seems to me that any such limit must of necessity be artificial, and therefore unsatisfactory. Until definite copulation of living flagellates has been seen, it is unwise to attempt to differentiate between various forms of parasites and to ascribe sex or lack of sex to any particular phase of the organism.

Berliner (1909) and others have asserted, but not proved, that autogamy results in cyst production. As autogamy is not a necessary accompaniment of encystment, I see no reason why its aid should be invoked here, and consider that unless it can be proven, it is well not to make such assertions. Preceding cyst formation, there are two successive divisions of *H. jaculum*, whereby small daughter parasites are produced. This *decrease* in the size of the parasite is opposed to the idea of autogamy. I have never seen any form either of autogamy or of sexual dimorphism in *H. jaculum, H. muscae domesticae, H. culicis* or *H. vespae*, n.sp.[1], all of which I have observed in the living condition. Nor am I alone in this. Neither Léger nor Patton have seen clearly defined sexuality in the Herpetomonads that they investigated.

Mode of Infection.

The mode of infection of *Nepa cinerea* by *H. jaculum* may be casual or hereditary or both. Regarding the first method, careful attention has been given to the habits of *Nepa cinerea*, both in their natural breeding grounds—a matter of some difficulty—and also in captivity in an aquarium.

The *Nepa* are found to breed among leaves of aquatic plants, the chief being *Lemna major, Elodea canadensis, Potamogeton crispus* (in one locality), *Veronica beccabunga* and *Hydrocharis morsus ranae.* Their faeces are voided on the leaves of these plants, forming blackish patches and having a peculiar odour in mass. Examination of the faeces showed flagellate Herpetomonads and cysts. Young *Nepa* move about among these leaves and suck up the faeces with the moisture. Individual *Nepa* so engaged have been watched carefully, seized quickly and the mouth region of the bug pressed against a clean slide. The small amount of darkish material so obtained, when stained and examined microscopically is found to contain cysts. Immediate dissection of the crop of the same *Nepa* also has shown that cysts are present in its contents, while in the case of very young nymphs, no parasites were present in the rest of the crop, so that the ingested cysts would cause a primary infection of the nymph.

This certainly is definite evidence of casual infection, agreeing therein with what Patton (1908–9) has described for *H. lygaei* and *H. muscae domesticae* where the same method of infection prevails. Berliner (1909) has noted this method of infection also in connection with *H. jaculum* but does not give experimental proof of his statement that the *Nepa* take up the cysts of the flagellate by ingesting the faeces of their fellows.

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[1] I wish to record the occurrence of a new Herpetomonad, parasitic in the alimentary tract of the hornet, *Vespa crabro*. I have named this flagellate *Herpetomonas vespae*, and hope to publish an account of it shortly.

Another method of infection is that due to the cannibalistic habit of *Nepa cinerea.* These insects are not only blood-suckers and coprophagous, but I have seen young nymphs and weakly adults seized and killed by stronger *Nepa,* and have seen them drag out and devour the entire viscera of their victims. The attacks were usually made at the metathorax, and the viscera were drawn out, either from the wound or else from the anus. As this feeding habit is common, there is the possibility of the *Nepa* acquiring all stages of *Herpetomonas jaculum* by way of its food.

The Evolution of Hereditary Infection.

The occurrence of *Herpetomonas jaculum* in the ovaries of *Nepa cinerea* has already been mentioned. The parasites have been seen to pass through the wall of the gut near the ovaries and to penetrate them. The Herpetomonads having entered the ovaries rapidly lose their flagella (Pl. V, Fig. 46) and become very broad and large (Figs. 47, 48). Occasionally groups of these non-flagellated forms (Fig. 49) are found in preparations. The nucleus and blepharoplast of each *Herpetomonas* is fairly distinct. Large granules of chromatin are present in the general body protoplasm, and the latter is much more alveolar than in the forms in the gut (Figs. 46—49). It seems, therefore, that the flagellates degenerate in the ovaries of *Nepa cinerea.* Though I have examined many ova and embryos of the host, I have failed to find infected ones. Nevertheless, the occurrence of the parasites in the ovaries seems to be a step on the way to hereditary infection. But the Herpetomonads have not yet fully adapted themselves to this form of parasitism, consequently degeneration ensues and the Herpetomonads die. The phenomenon of hereditary infection "in the making" has also been observed by me in the case of *Crithidia gerridis,* while *Crithidia melophagia,* on the other hand, is capable of infecting the ova of *Melophagus ovinus.*

Environmental Effects.

In the course of the investigation of *H. jaculum,* some experiments were performed on the effects of certain changes of environment on the parasite.

1. *Effects of Light.*

The movements of the Herpetomonads were slowed in proportion as the intensity of ordinary white light was decreased. Green light also caused the organism to move more slowly.

2. *Effect of Temperature.*

In tap-water at 15° C. the organisms moved rapidly; at 18° C. their movements were more active, although the rise in temperature was slight.

3. *Effects of Various Media.*

(i) *Tap-water.* The parasites behaved as in the gut of the host and lived for several hours.

(ii) 0·75 °/₀ NaCl. The flagellates died more rapidly in this liquid than in tap-water.

(iii) *Glycerine.* The movements of Herpetomonads were slowed by dilute glycerine.

(iv) 1 °/₀ *acetic acid* killed the flagellates, which appeared to swell slowly and then burst.

(v) 1 °/₀ *caustic potash* killed the parasites rapidly. Their bodies dissolved very slowly.

(vi) A medium composed of the gut contents of other *Nepa,* recently fed on larvae of *Chironomus,* caused starved Herpetomonads placed therein to develop granules in their cytoplasm.

(vii) *Human blood* derived from a pricked finger, likewise fish and amphibian blood, when mixed with the parasites, had the effect of causing growth, rapid longitudinal division and increase of granules in the protoplasm of the Herpetomonads.

(viii) A medium composed of crushed *Culex* larvae in water, when mixed with *H. jaculum,* caused the flagellates to divide, but the division was not so frequent nor so rapid as recorded under (vii).

Experiments (vii) and (viii) suggest that division of the flagellate Herpetomonad takes place rapidly under natural conditions, after ingestion of blood by the host.

General Remarks.

Herpetomonas jaculum (Léger) is a parasite whose complete life is passed within the alimentary tract of *Nepa cinerea.* Its long body, blunt flagellated end and single flagellum, in addition to the three phases of its existence, place the parasite in the genus *Herpetomonas* as originally constituted. Saville Kent (1881) created the genus

Herpetomonas for a parasite found in the alimentary tract of the common house-fly, the parasite having been previously described by Burnett (1851) as a *Bodo*. At that time, the genus *Herpetomonas* was little known, and the existence even of a nucleus in the parasite was doubtful, but one flagellum was regarded as a constant feature. Since Kent established the genus, comparatively many parasites have been placed therein, but the life-cycle of most of them is incompletely known, the flagellate stage of existence usually being the only one described, and that often imperfectly, owing to too much reliance having been placed on stained specimens to the exclusion of valuable facts that could be obtained by the study of the living organism. When the development and life-cycle are completely followed in the living organisms, misinterpretation of morphological details is rendered far less possible.

Léger (1903–4) worked on various parasites which he placed in the genus *Herpetomonas,* considering them uni-flagellate. One of these was *H. jaculum* from *Nepa cinerea,* the flagellate stages of which were described with characteristic painstaking detail. These flagellated forms were termed by him "les formes monadiennes." A second form of the parasite, "la forme grégarinienne" is also described as being derived from the first form. As these occurred in old *Nepa,* in the lower portions of the gut, they would now be considered as forms in which the transition to the post-flagellate stage had begun.

Prowazek (1904) gave a long and involved account of *Herpetomonas muscae domesticae.* He described the flagellate stage of the parasite only, and his observations commenced on the parasite in this condition. Hence owing to lack of knowledge of the development of the parasite, there were many chances of errors of interpretation, some of which errors actually occurred. This account of Prowazek's has caused much argument and has resulted in great confusion regarding the systematic position of organisms formerly considered Herpetomonads, but which must be assigned to other genera should Prowazek's work be accepted. Prowazek declares that *H. muscae domesticae* has two flagella, these being united by means of a narrow membrane. He also describes copulation between sexual forms, this being preceded by a most complicated series of reducing divisions of both nucleus and blepharoplast, and also reduction of the flagellar apparatus. The copulation results in a cyst which is voided with the faeces.

The subject of this double flagellum will be referred to later (p. 384).

Patton (VII. 1907) in his account of *Herpetomonas culicis* points out the resemblances existing between stages of this parasite and those of

Kala-azar. He was the first to demonstrate the three phases of the life-history of Herpetomonads, and further stated that before the bi-flagellate nature of *H. muscae domesticae* could be accepted, the entire development of the flagellate must be considered. In the same year (1907), Patton gave a brief résumé of the insect flagellates of Madras, pointing out the occurrence of Herpetomonad parasites in no less than eight insects that he had personally investigated.

In 1908, Patton described *H. lygaei*, occurring in the bug *Lygaeus militaris*. Its full life-cycle was given in detail, and the reasons for placing it in the genus *Herpetomonas* were also set forth. In this same paper (1908, p. 12), Patton stated that he had himself followed the development of *H. muscae domesticae*, and that "the appearance of a double flagellum...can only represent the commencing division of the flagellate." Donovan (1909) has confirmed Patton's statements.

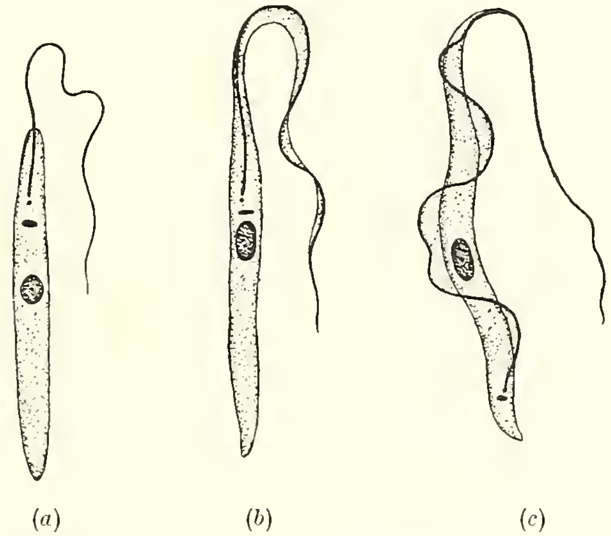
From my own examination of house-flies infected with *H. muscae domesticae* (see p. 374) I can confirm Patton's statements. During my researches, I have watched the flagellum of the living *H. muscae domesticae* divide into two in exactly the same manner as in *H. jaculum*, *H. culicis*, *Crithidia gerridis* and *C. melophagia*.

It would appear, then, from more recent work, that Prowazek's interpretation of the double flagellum as a *constant* feature of the type species of *Herpetomonas* is not accurate.

Chatton and Alilaire (1908) published a short note on a flagellate from *Drosophila confusa*, called *Leptomonas* (*Herpetomonas*) *drosophilae*. These authors have accepted Prowazek's characterisation of *H. muscae domesticae* as bi-flagellate. The development of *Leptomonas drosophilae* is not completely worked out, though it is confidently asserted that the infection of the fly is hereditary. On account of the organism being aciculate and possessing a single flagellum, Chatton and Alilaire state that they re-institute the generic name *Leptomonas* for their parasite, that name having been first given by Kent to a flagellate described from a Nematode, *Trilobus sp.*

Roubaud (1908) accepts the definition of *Leptomonas* given by Chatton and Alilaire.

Berliner (1909), investigating *H. jaculum*, considers it bi-flagellate, but states that the parasite begins by having a single flagellum. He considers that a second flagellum grows out from near the root of the first. Berliner therefore follows Chatton and Alilaire, but also places the genus *Crithidia* in the genus *Leptomonas*, so that *Crithidia gerridis* becomes *Leptomonas gerridis*; *C. melophagia*, *L. melophagia*, etc. This compounding of genera cannot be commended. *Crithidia* are very distinct from *Herpetomonas*, the undulating membrane and the anteriorly tapering body of the adult *Crithidia* being quite unlike the appearance of the *Herpetomonas*, while the nucleus and blepharoplast of the *Crithidia* are nearer together than those of *Herpetomonas* (Text-fig. 1, *a*, *b*). The flagellum also is somewhat different in the two, for in *Crithidia*, it is more of the nature of the chromatic border of the undulating membrane of a Trypanosome (Text-fig. 1, *c*), while in *Herpetomonas*, the flagellum is free and no membrane is present. There is also a distinct difference in the movements of the two organisms, sufficient to differentiate between the parasites in general observation. These points surely should be sufficient to deter anyone from placing such different organisms in one and the same genus. Personally, I believe that the genus *Herpetomonas*[1] should be retained, as it was originally constituted, for flagellates each possessing one flagellum and



Text-fig. 1. Diagrams of (*a*) *Herpetomonas*, (*b*) *Crithidia*, (*c*) *Trypanosoma*—to show the chief morphological differences between these genera, especially as regards the flagellum and undulating membrane.

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[1] An interesting discovery has been made by Lafont (VI. 1909) of a flagellate, *Herpetomonas* (*Leptomonas*) *davidi*, parasitic in the latex of *Euphorbia pilulifera*, a shrub common throughout the tropics. The parasitised plants, suffering from a condition which the author terms "flagellosis," lose their leaves, and their growth suffers.

Vincent has found the flagellate in *Euphorbia thymifolia* as well as in *E. pilulifera* in Réunion. The plants were infested with small Hemiptera, and it is suggested that these are the carriers of infection. Donovan (XI. 1909) has also found the flagellate in *E. pilulifera* of Madras.

I have had the opportunity of examining a preparation, sent from Madras, of the flagellate from *Euphorbia*, and consider it a typical, uni-flagellate *Herpetomonas*.

no undulating membrane, such as *H. lygaei, H. jaculum, H. culicis* and *H. muscae domesticae.*

Regarding the systematic position of the Herpetomonads among the flagellates, consideration of their life-cycle with its three phases, places them with the *Crithidia*, and the latter are united on morphological grounds with the Trypanosomes. It is possible that when the full life-cycle of the latter organisms is known, it will be on the Crithidial plan, showing pre- and post-flagellate phases.

The genus *Herpetomonas* would then be the lowest in organisation of these three great genera. In *Herpetomonas* an undulating membrane does not exist (Text-fig. 1, *a*). In *Crithidia* it is present but is relatively small (Text-fig. 1, *b*), while the Trypanosomes possess well-marked membranes (Text-fig. 1, *c*). The blepharoplast is in the anterior (pre-nuclear) region of both *Herpetomonas* and *Crithidia.* The flagellum arises near it, and in *Crithidia*, as it passes outwards, it draws out the body in the form of a membrane which becomes lengthened as the body elongates.

The family *Trypanosomatidae*, then, contains forms varying somewhat in detail among themselves, but having the same general principle underlying their organisation, the simplest members being that to which *H. jaculum* belongs, the Herpetomonads.

With regard to the phylogeny of the *Trypanosomatidae*, I feel that there is far too little evidence at present to justify hypotheses on the subject. (See Patton and Strickland (1908), p. 340.)

In conclusion, I would again point out the need for the study of the living organism, at every stage of its life-history. While *H. jaculum* is purely a parasite of an arthropod, its study, like that of *H. muscae domesticae*, is a clue to the mode of life and reproductive processes of other, possibly pathogenic, organisms to which it is nearly allied.

Summary.

(1) *Herpetomonas jaculum* is a parasite of *Nepa cinerea*, occurring in the alimentary tract of its host.

(2) The life-history of the parasite may be conveniently divided into three stages, the pre-flagellate, flagellate and post-flagellate stages, which gradually merge into one another.

(3) The movements of the parasite are less flexible than those of *Crithidia*, as Herpetomonads have no undulating membrane. The flagellum is the most active agent in effecting motion.

(4) The pre-flagellate stages of the parasite (Pl. V, Figs. 1—18) are best observed in the crops of nymphs of *Nepa cinerea.* The parasites at first are oval (Figs. 1—6). They vary in size from about 4 μ to 5 μ long and from 2 μ to 2·5 μ broad. They show nucleus and blepharoplast, and may divide longitudinally before flagella are acquired (Figs. 2—4). The flagellum of each parasite arises from a region near to the blepharoplast but not directly from it (Figs. 5—9).

(5) The flagellate stage of the organism (Pl. V, Figs. 19—36) is that best known. *H. jaculum* is from 13 μ to 33 μ long and from 1 μ to 4 μ broad, the size varying according to the recency or otherwise of longitudinal division. Myonemes (Figs. 20, 21, 38) are present on the body. The flagellum is at least as long again as the body. The nucleus contains a number of grains of chromatin (Figs. 28, 34), sometimes in the form of eight large grains (Figs. 34, 36), sometimes as very fine granules (Figs. 22, 31). The blepharoplast is in the anterior, pre-nuclear, region of the parasite, and is usually rod-like (Figs. 19, 20, 21). The single flagellum (Fig. 19) arises near it but not from it. A basal granule (Figs. 22, 33, 34) is present at or near the origin of the flagellum. Chromidia are present as scattered granules in the body (Figs. 28, 32, 33).

(6) The post-flagellate stage is the form assumed by the parasite for life outside the body of the host. Preceding encystment, the organism divides twice longitudinally, giving rise to four daughter forms (Pl. V, Figs. 51—54) each of which ultimately loses its flagellum, rounds itself off and forms a cyst (Figs. 57—68). These cysts are from 2·5 μ to 4·5 μ long and from 1·4 μ to 2·6 μ broad. They occur in the rectum of *Nepa cinerea* and are voided with the faeces, being ingested later by other bugs.

(7) Longitudinal division is the common method of multiplication of *H. jaculum.* The flagellum may divide precociously, but usually division is initiated by constriction of the blepharoplast (Pl. V, Fig. 37) almost simultaneously with division of the flagellum and followed by that of the nucleus (Figs. 39, 40). A split occurs (Figs. 40—42) and the active movements of the two flagella aid in the divergence of the daughter organisms (Figs. 43, 44), which ultimately separate.

(8) I have no evidence whatever for ascribing sex to any form of *Herpetomonas,* but consider the occurrence of long and short and of thin and stout forms to be explicable as the results of growth and division. Also, I have shown experimentally that richly granular protoplasm is the result of a physiological condition and is not necessarily fixed as an attribute of the female sex.

(9) One mode of infection has been proved experimentally in the laboratory and also observed at the breeding grounds of the *Nepa*. Cysts voided in infected faeces are swallowed by other *Nepa* in the adult and nymphal stages. The crops of such nymphs on dissection were found to contain cysts, whereas no flagellates were present in other parts of the gut, so that primary infection occurred here in the crops of the nymphs.

Cannibalism of *Nepa cinerea* whereby other *Nepa* are devoured is also responsible for the spread of *H. jaculum*.

I have no definite evidence of hereditary infection, although I have found flagellate and post-flagellate forms (Pl. V, Figs. 46—49) in the ovaries of the host. The parasites were not found in the eggs. The occurrence of parasites in the ovaries may be regarded as a stage in the evolution of hereditary infection.

(10) Various environmental effects have been studied, the most important observation being that fresh food appears to stimulate the parasites and to cause their rapid division.

(11) The generic name *Herpetomonas* should be retained, as originally constituted, for parasites having but one flagellum and no undulating membrane (see pp. 383 *et seq.*).

(12) I wish to record the occurrence of a new species of *Herpetomonas, H. vespae,* from the alimentary tract of the hornet, *Vespa crabro*.

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EXPLANATION OF PLATE V.

All figures were outlined with an Abbé-Zeiss camera lucida, using a 2 mm. apochromatic (Zeiss) or $\frac{1}{12}$ inch achromatic (Zeiss) objective and compensating oculars 8 and 12 of Zeiss.

The magnification is, in all cases, approximately 1300 diameters, except Figs. 34 and 36 which are approximately 1950 diameters.

Figs. 1—18. Pre-flagellate stages.

Fig. 1. Oval pre-flagellate with large nucleus and blepharoplast. Flagellar area below rod-like blepharoplast. Delafield's haematoxylin.

Fig. 2. Stage in the division of the pre-flagellate. The Herpetomonad has elongated, the blepharoplast is divided and the nucleus partly divided. Chromatin staining area above blepharoplasts. Delafield.

Fig. 3. Further stage in division. Both nucleus and blepharoplast are divided, and a vacuole is forming in the mid-line. Division of body commenced. Thionin.

Fig. 4. Still later division. Fission of body has continued. Flagellar precursor seen. Delafield.

Fig. 5. Herpetomonad whose flagellum has begun to appear. Delafield.

Fig. 6. Parasite with flagellum at edge of body. Giemsa.

Fig. 7. Form showing flagellum just clear of the body. Thionin.

Fig. 8. Rounded parasite with large blepharoplast and basal granule, near which the thick flagellum arises. Iron-haematoxylin.

Fig. 9. Parasite with flagellum lying very close to edge of the body. Giemsa.

Fig. 10. Large pre-flagellate of slightly irregular contour. Very alveolar protoplasm. Large chromatin granules in the nucleus. Giemsa.

Fig. 11. Parasite with flagellum free from body. Large, somewhat oval blepharoplast present. Thionin.

Fig. 12. Large pre-flagellate with basal granule and rounded nucleus. Delafield.

Fig. 13. Large form, showing flagellum just extended beyond the body. Thionin.

Figs. 14, 15. Somewhat pear-shaped parasites with free flagella. Their anterior ends are somewhat attenuate. Giemsa.

Fig. 16. Elongating pre-flagellate with round nucleus and curved blepharoplast. Twort.

Fig. 17. More elongate parasite. Chromidia present in the posterior end of the body. Delafield.

Fig. 18. A division rosette of pre-flagellates. One of these has again divided into two, which have not yet acquired flagella. The undivided form has grown a flagellum and has elongated posteriorly, being practically in the full flagellate condition. Giemsa.

Figs. 19—36. Illustrate the flagellate stage.

Fig. 19. Ordinary flagellate. Posterior end somewhat pointed. Large nucleus and well-marked blepharoplast present. Blepharoplast slightly inclined to the long axis of the parasite. Delafield.

Fig. 20. Somewhat broad parasite, commonly occurring. Myonemes seen on the body. Blepharoplast extending part way across the body. Delafield.

Fig. 21. Larger form with well-marked myonemes. Vesicular nucleus with a few grains of chromatin. Blepharoplast across almost the entire width of body. Vacuole present in posterior end. Thionin.

Fig. 22. Parasite showing the arrangement of granules in the protoplasm suggestive of a cytopharynx. Vesicular nucleus. Basal granule well seen. Giemsa.

Fig. 23. *Herpetomonas* with body twisted on itself. Richly granular protoplasm, thick flagellum and basal granule seen. Thionin.

Fig. 24. Flagellate showing nucleus with large masses of chromatin. Blepharoplast is vertical, an uncommon position. Delafield.

Fig. 25. Very broad parasite, with short flagellum, vesicular nucleus and posterior vacuole. Thionin.

Fig. 26. Small *Herpetomonas* somewhat tapering at both ends. Giemsa.

Fig. 27. Form with a very tapering posterior end and oblique blepharoplast. Iron-haematoxylin.

Fig. 28. Parasite showing well-marked posterior vacuole, isolated large chromidia and basal granule. Delafield.

Fig. 29. Flagellate with thick flagellum. Giemsa.

Fig. 30. *Herpetomonas* with large nucleus and curved blepharoplast. Thionin.

Fig. 31. Small *Herpetomonas* with round nucleus, curved blepharoplast and isolated chromidia. Giemsa.

Fig. 32. Parasite having nucleus with large chromatin granules. Blepharoplast inclined to the long axis of the body. Tapering posterior extremity. Delafield.

Fig. 33. *Herpetomonas* showing nucleus with distinct chromatin granules, large blepharoplast, chromidia in the posterior end, and thick flagellum. Thionin.

Fig. 34. Specimen showing large chromatin masses in the nucleus, curved blepharoplast, basal granule, large, thick, tapering flagellum, vacuole at the posterior end of the body and chromidia. Delafield. × 1950.

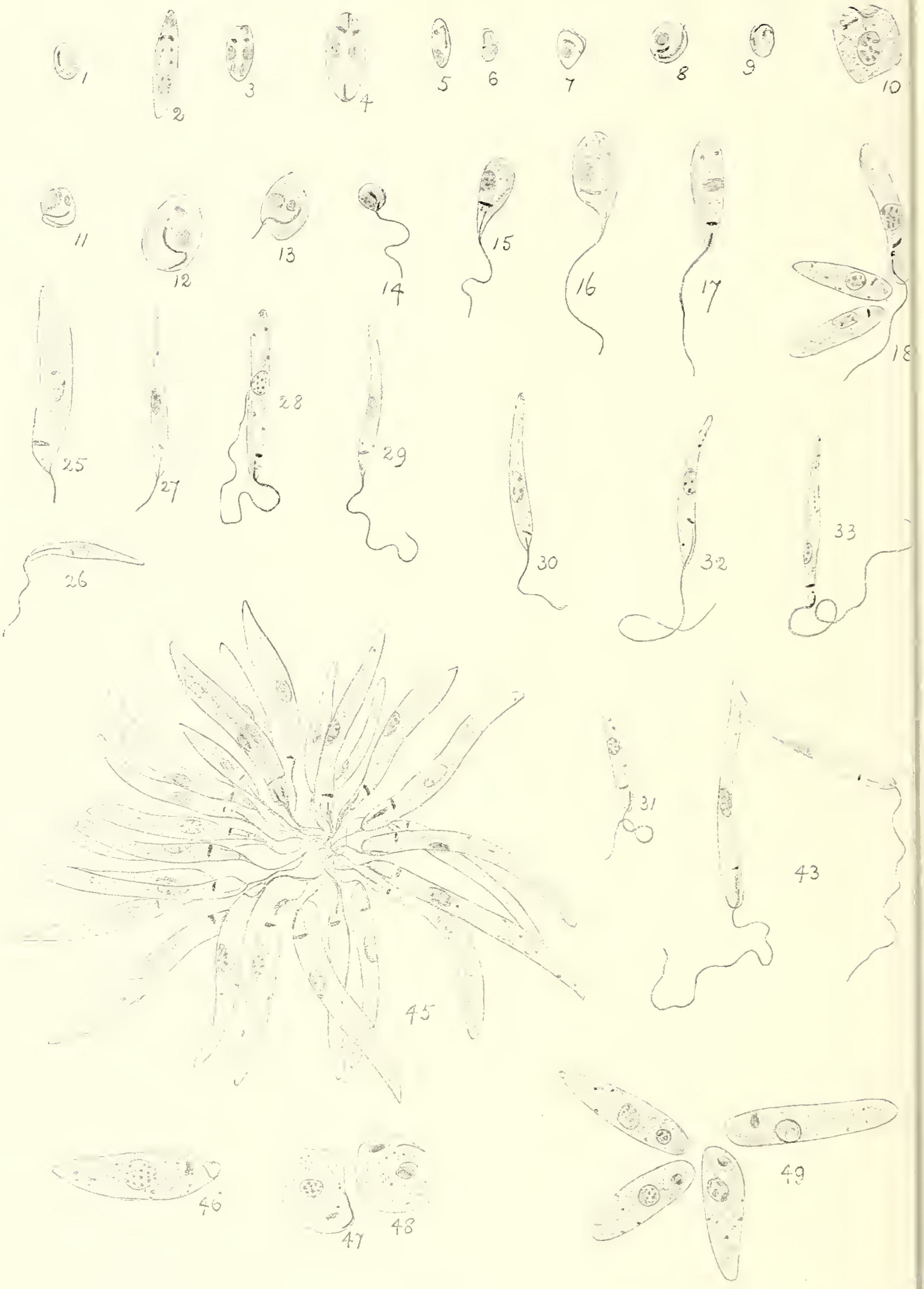
Fig. 35. Very narrow *Herpetomonas*—the product of repeated longitudinal division, but showing typical structure. Safranin.

Fig. 36. Parasite similar to the one shown in Fig. 34, but the blepharoplast is oval and the chromatin granules more regularly disposed in the nucleus. Delafield. × 1950.

Figs. 37—44. Stages in the division of flagellates.

Fig. 37. Early stage of division showing constricted blepharoplast and nucleus and precocious division of the root of the flagellum. Delafield.

Fig. 38. Division form, nucleus and blepharoplast much as in Fig. 37, but with the flagellum still more divided. Myonemes showing well on the body. Giemsa.



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HERPETOMONAS

PLATE V

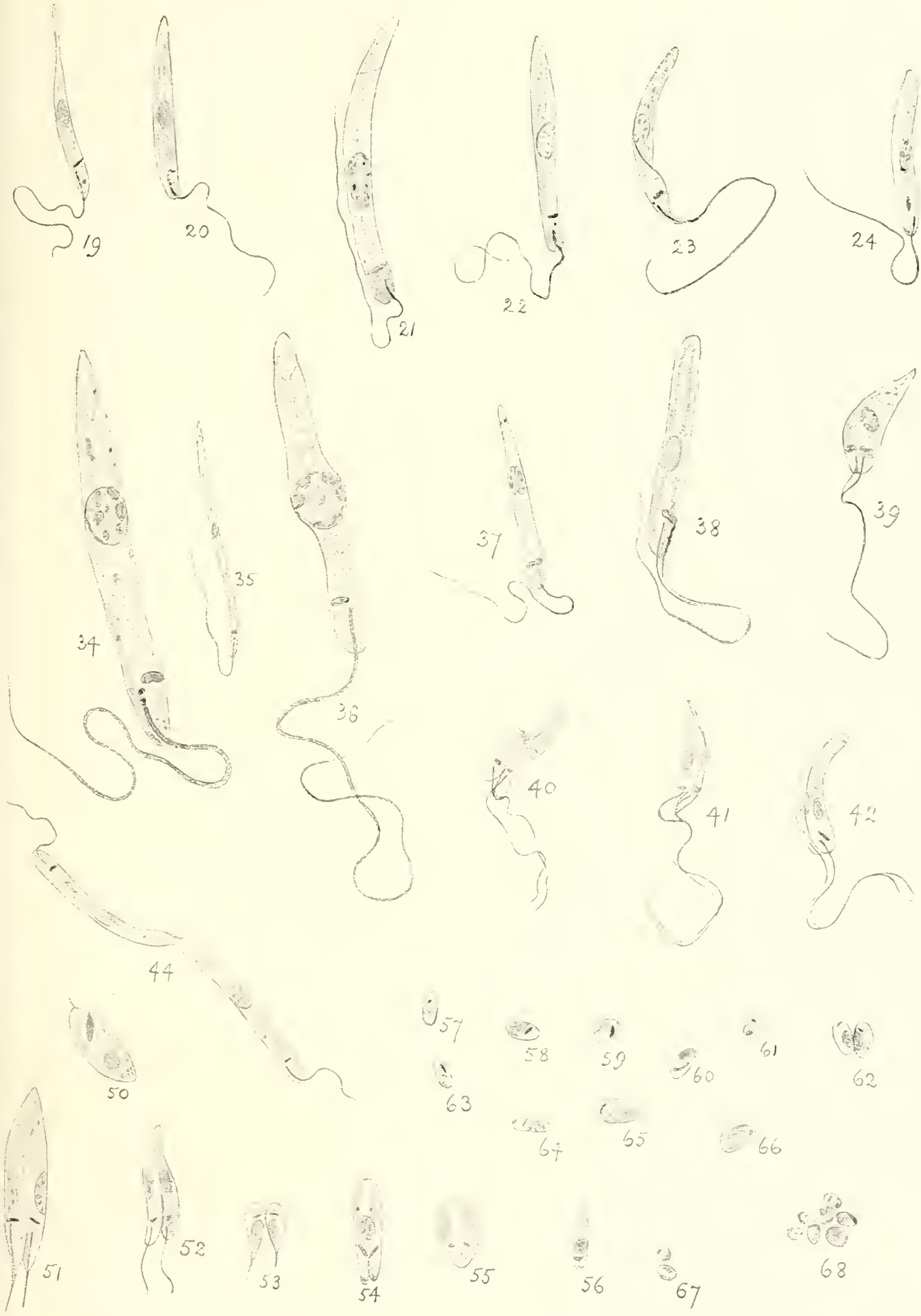

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Fig. 39. Early stage of division seen from the side. Blepharoplast divided. Nucleus constricted. The "double flagellum" stage. Giemsa.

Fig. 40. Further stage in division. Nucleus, blepharoplast and flagellum all divided. Delafield.

Figs. 41, 42. Same stage as in Fig. 40, but seen from the side. Thionin.

Fig. 43. Daughter organisms widely diverging from one another. Bodies almost separated. Giemsa.

Fig. 44. Division almost completed. The organisms are in a straight line with one another, and final separation is about to occur. Delafield.

Fig. 45. Aggregation rosette of *Herpetomonas jaculum*. Flagella attached to food particle. Delafield.

Figs. 46—49. *Herpetomonas* from the ovaries of *Nepa cinerea*.

Fig. 46. Large broad form with alveolar protoplasm, nucleus with fine granules, and many chromidia. Flagellum very small. Giemsa.

Figs. 47, 48. Further stage in degeneration of the *Herpetomonas* in the ovary. Many chromidia scattered in protoplasm. Thionin.

Fig. 49. Group of four, non-flagellated forms from the ovaries. Very alveolar protoplasm. Chromidia. Delafield.

Figs. 50—68. Post-flagellate stages.

Fig. 50. Short, broad parasite whose flagellum has almost disappeared. Giemsa.

Figs. 51, 52. Form showing first division prior to encystment. Fairly large granules in nucleus. Delafield.

Fig. 53. Form after second division prior to encystment. Flagellum as such has disappeared. Thionin.

Fig. 54. Partly divided form that has no flagellum. Thionin.

Fig. 55. Almost divided parasite with clear, central vacuole at the line of separation. Delafield.

Fig. 56. Small form produced by the division of such forms as those shown in Figs. 54, 55. Delafield.

Figs. 57—66. A series of cysts (post-flagellates) produced by the rounding of small forms. Fig. 62 shows two daughter-cysts side by side. There is much variation in the size of the cysts, but each shows a nucleus and blepharoplast.

Fig. 67. Two small cysts. Delafield.

Fig. 68. Group of cysts. Common in the rectum. Safranin.

THE SPIROCHAETES FOUND IN THE CRYSTALLINE STYLE OF *TAPES AUREUS*: A STUDY IN MORPHOLOGICAL VARIATION.

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(With Plate VI and 2 Text-figures.)

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Introduction.

One of the earliest known Spirochaetes from Lamellibranchs was that briefly described by Certes in 1882 from the crystalline style of the oyster. Certes named the parasite *Trypanosoma balbianii*. In 1891 the same worker recorded the occurrence of the organism in two species of *Tapes*, namely *T. decussatus* and *T. pullaster*. Laveran and Mesnil in 1901 showed that *Tr. balbianii* probably belonged to the Spirochaetes. The organism is now known as *Spirochaeta balbianii*.

While on a visit to Banyuls-sur-mer (Pyrénées orientales) in March and April 1908, I was enabled to examine living specimens of *Tapes aureus*[1] through the kindness of Professor Pruvot and Dr Racovitza. The *Tapes*, which are edible, were sent to the Laboratoire Arago at Banyuls from La Nouvelle, and were locally known as "les clovisses." The specimens in the first consignment of *T. aureus* were not infected with Spirochaetes, but the *Tapes* of a second consignment nearly all contained Spirochaetes in their crystalline styles. The alimentary canals of the *Tapes* were carefully dissected fresh, and a detailed examination of the crystalline styles was made. A large and well-developed style was found in nearly every *Tapes* examined. The infection with Spirochaetes inside the style was often intense, so that the interior of the gelatinous style was literally "alive," affording a most interesting sight, especially to those who had not seen living Spirochaetes in Lamellibranchs before.

Owing to pressure of other work, the examination of the stained preparations was deferred, and was done in the Quick Laboratory, Cambridge.

Technique.

The *Tapes* were kept alive in aquaria containing sea-water at the marine laboratory, and were used quite fresh. Crystalline styles containing Spirochaetes were divided into small portions, mixed with a little filtered sea-water and teased on slides or cover-slips. For observations of living Spirochaetes, wet preparations were made, the cover-glass being sealed with vaseline. Preparations intended for staining were fixed wet with osmic vapour or with alcoholic Bouin's liquid (picro-formol-acetic), graded through increasing strengths of alcohols and stained with iron-haematoxylin, Delafield's haematoxylin, thionin or gentian violet. A few preparations were stained with Giemsa's solution, and with Twort's stain (neutral red and Lichtgrün). The various preparations were carefully compared.

Other preparations were fixed fresh with corrosive-acetic alcohol and then treated with gold-chloride in order to study the action of this reagent on the contractile fibrils or myonemes of Spirochaetes. Some Spirochaetes were also mounted in methyl alcohol, in a further endeavour to study myonemes. The results obtained after treatment of the parasites with gold-chloride or with methyl alcohol were not superior to those obtained after using somewhat less elaborate methods.

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[1] *Tapes aureus* Forb. et Hly. (*Venus aurea* Gm., *V. sinuata* Gm., *V. amygdala* Meuschen.) See J. Victor Carus, *Prodromus Faunae Mediterraneae* (1889–93), II. pp. 126—7.

Observations on the Living Spirochaetes of Tapes aureus.

The Spirochaetes of *Tapes aureus* were examined alive in a medium consisting of crystalline style substance to which a little filtered sea-water had been added. Their movements were *very* active, and similar to those of *S. balbianii* and *S. anodontae,* in that they may be resolved "into at least two components: (i) an undulatory flexion of the body, mainly for progression, and (ii) a spiral or corkscrew movement of the body as a whole, due to the winding of the membrane." (Fantham, I. 1908, p. 17; compare also VI. 1907, p. 495.)

While examining the rapidly moving living specimens, it was evident that considerable variation existed among the Spirochaetes from the style of any given *Tapes aureus,* and that detailed examination of stained preparations would be necessary to determine whether more than one species of Spirochaete was present, or only a number of varieties of the same species.

Both longitudinal and transverse division were seen to occur in the living Spirochaetes. In longitudinal division the daughter individuals were observed to diverge gradually, like the legs of a compass, while still remaining undivided at one end. In transverse division the parent form usually elongated slightly and divided about a central node. Waves travelled in opposite directions from the ends of the organism about to divide towards its middle, and then back again. A series of such waves ensued, and finally the parent organism parted in the middle. The process of transverse division in living Spirochaetes has been described by Miss Porter and myself (1909) for *S. anodontae, S. recurrentis* and *S. duttoni.*

Sometimes Spirochaetes of *Tapes* were seen to bend over and loop in the middle (cf. Pl. VI, Figs. 45—48) forming a U, the halves of which sometimes intertwined. These looping forms are perhaps suggestive of division, but in no case was division seen to occur. Usually the Spirochaetes untwined and swam away. Similar phenomena have been described and figured for *S. balbianii* and *S. anodontae* by Miss Porter (1909).

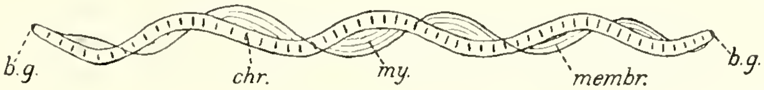
A few Spirochaetes were seen to coil themselves spirally, either at one end or in the middle. Later the Spirochaetes uncoiled and swam away. Parasites fixed in the coiled position and then stained (Pl. VI, Fig. 53) suggest encystment. Study of the living organism shows that true encystment does not occur.

Agglutination was not seen in the Spirochaetes of *Tapes aureus.*

General Morphology.

The structure of the Spirochaetes of various Lamellibranchs has been more or less fully described by Laveran and Mesnil (1901), Perrin (1906), Keysselitz (1906—7), Swellengrebel (1907), Fantham (1907—8), Gonder (1908), Schellack (1909) and others. I made a detailed study of two such Spirochaetes *S. balbianii* and *S. anodontae* in 1906—7. It will not be necessary to repeat in detail an account of the typical structure of a Spirochaete as seen in specimens from the gut of *Tapes aureus.* A summary of the leading features will suffice.

A Spirochaete is covered externally by a flexible periplast, which, when stained under favourable conditions, shows contractile threads or myonemes (Text-fig. 1). Miss Porter (1909), using dark-ground illumination, has actually seen pulsations travelling along the myoneme fibrils in *living S. anodontae.* Internal to the periplast is the nuclear apparatus, which during the trophic phase consists of transverse bars of chromatin, as judged by staining reactions. These chromatin bars are really joined obliquely by achromatic connections (Pl. VI, Fig. 39) —usually better seen during division. The whole nuclear system forms a somewhat flattened helix, the turns of which closely approximate to the external periplast. During division the chromatin is somewhat rearranged, for some stainable granules (presumably of chromatin) migrate into the more achromatic, oblique portions of the spiral, and so the helix is more easily stained as a whole.



Text-fig. 1. Diagram of a typical Spirochaete (*e.g. S. balbianii*) with rounded ends, from marine Lamellibranchs.

b.g. = basal granule.
chr. = transverse bar of chromatin.
membr. = membrane with myonemes (*my.*) approximately longitudinally disposed.

The rest of the substance of the body of a Spirochaete seems to be of a cytoplasmic nature, possibly somewhat alveolar. As I have previously stated (I. 1908, p. 13), the cytoplasm does not usually stain at all deeply with most "plasma" stains, Lichtgrün excepted.

At each end of the organism is a basal granule ("calotte" of Swellengrebel) which stains like chromatin. Gonder (1908—9) has recently found basal granules in *S. pinnae.*

Each Spirochaete possesses a membrane (the "undulatory membrane" of many authors, or the "periplast appendix and chromatic band" of Swellengrebel), which is a spirally coiled lateral extension of the periplast, and contains myonemes. The membrane is contractile, and is a locomotor organella.

The relation of the membrane to the body of the Spirochaete is seen on examining sections of the parasites (Pl. VI, Fig. 54), obtained on cutting sections of the crystalline style of an infected Lamellibranch (cf. Fantham, I. 1908, p. 30, Text-fig. 6). In cross-section the periplast of the Spirochaetes is somewhat refractile and stains rather deeply. These characteristics of the periplast are probably due to the presence therein of a chitinoid substance. The periplast is firm, yet very flexible, strength and power to bend both being due to its physical and chemical nature.

The Various Spirochaetes of Tapes aureus.

On examining a preparation from an infected style of *T. aureus* (*Tapes*, No. 1), fixed wet with Bouin's liquid and stained with iron-haematoxylin, three forms of Spirochaetes could usually be seen in any one field of the microscope. Although intermediate forms occur, we may for convenience' sake divide the prevailing forms into three groups, A, B, and C:—

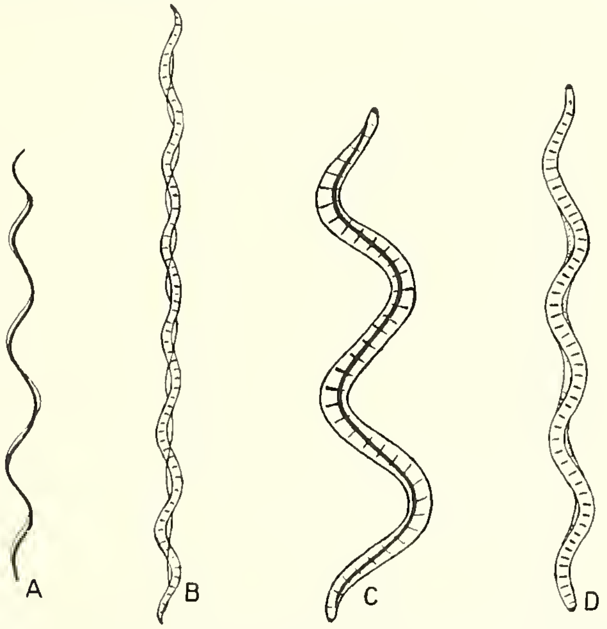
Group A includes very thin, undulatory Spirochaetes, with well-marked membranes, and bodies from 30μ to 60μ long, and about $0{\cdot}3\mu$ broad (Text-fig. 2 A, Pl. VI, Figs. 1—5).

Group B includes long, somewhat thicker Spirochaetes, usually with pointed ends, well-marked edge to the membrane and a tendency to have flattened spirals (Text-fig. 2 B, Pl. VI, Figs. 13, 15—21). These parasites are from 50μ to 90μ long, and 1μ to $1{\cdot}3\mu$ broad.

Group C includes shorter, broader forms (Text-fig. 2 C, Pl. VI, Figs. 35—37), with membrane sometimes well-marked, sometimes closely contracted around the body, which is from 50μ to 70μ in length and about 2μ in breadth. Usually only a few waves, of relatively large amplitude, occurred along the body.

This specimen *Tapes* (No. 1) contained many Spirochaetes of group A, a fair number of group B, and relatively few of group C.

The internal structure of chromatin granules or bars could be well seen in forms B and C, while in the very thin forms A it was with great difficulty, owing to the thinness of the organisms, that any internal structure could be demonstrated.



Text-fig. 2. Diagrams of various Spirochaetes found in the crystalline style of *Tapes aureus*.

A. Thin form, possessing clearly defined edge to its membrane.
B. Slightly broader, longer form, with pointed ends.
C. Broadest form, with contracted membrane, the edge of which is represented as a wavy, black line along the body. Ends tapering.
D. Spirochaete of medium breadth, with slightly tapering ends.

The groups A, B, C, D are really arbitrary ones, for various intermediate forms occur, so that they gradually merge into each other.

At first sight, it might be inferred from these observations that more than one species of Spirochaete was present in the crystalline style of the mollusc examined, or that sexual polymorphism was exhibited by the Spirochaetes. However, many more specimens of infected *T. aureus* were examined, when other Spirochaetes, chiefly intermediate in form, were found. These molluscs may, for convenience, be denoted successively as *Tapes* 2, 3, 4, 5, etc.

Thus, on examining a preparation from the style of *Tapes* 2 large numbers of another form of Spirochaete were found, which may be called group D.

Group D includes Spirochaetes which appeared rather longer than those in group C, with well-marked, expanded membrane showing myonemes, and often possessing slightly tapering ends (Text-fig. 2 D, Pl. VI, Figs. 29—32). These parasites are from 45μ to 70μ long and about 1·5μ broad. The waves, into which the body was thrown, were not quite of such great amplitude as those of the Spirochaetes of group C, so that Spirochaetes of group D are really of about the same length as those of group C, and not longer as appeared at first sight.

The Spirochaetes of group D, stained with Delafield's haematoxylin, are very like *S. balbianii* of the oyster, except for the slight tapering of the ends. Even this character of slightly tapering ends is seen in some of the drawings of *S. balbianii* of the oyster made by Certes in 1882, and by Borrel and Cernovodeanu in 1907.

On examining style-preparations from other specimens of *Tapes aureus*, different forms of Spirochaetes may be encountered, thus:

Tapes 3 contained intermediate forms, especially like those in group B (Pl. VI, Figs. 14, 22, 23), but possessing more rounded ends than those seen in *Tapes* 1. The stain used was Delafield's haematoxylin.

Tapes 4 contained Spirochaetes intermediate in thickness between A and B (Pl. VI, Figs. 6, 7). Other Spirochaetes on this preparation were long and slightly thicker than those of group B (Figs. 24, 27), with rounded ends, and bodies clearly showing transverse bars of chromatin internally. These Spirochaetes exactly resembled *S. balbianii* from the oyster. They were stained with Twort's stain.

Tapes 5 contained many long Spirochaetes with pointed ends belonging to group B. Two preparations were made from this mollusc. The first preparation was fixed wet with corrosive sublimate and stained with gold chloride. The second preparation was fixed wet with osmic vapour and stained with Giemsa's stain. The parasites in each were similar.

Tapes 6 contained a number of short, broad forms, with rounded ends (Pl. VI, Fig. 34), as seen in *S. balbianii* of the oyster. The parasites stained well with thionin.

Careful examination of a preparation from any one of these molluscs revealed the presence of a few Spirochaetes intermediate between those grouped under A, B, C and D. To summarise: On seven preparations all made by the wet method, from six different specimens of *Tapes aureus*, Spirochaetes of all lengths from 30μ to 100μ long, and all breadths from 0·3μ to 2·5μ could be found. Some had quite pointed ends, some possessed tapering ends, others were rounded, while yet others had their two ends differing from one another in their degree of roundness. In nearly every case the membrane and its edge could be clearly seen. On examining large numbers of Spirochaetes from many different *Tapes aureus*, however, intermediate forms exhibiting all gradations of length, breadth and degree of rounding of ends can be found (Pl. VI, Figs. 1—37). Several hundred *Tapes aureus* were examined, but without finding any substantially new variations of the Spirochaetes.

Division.

Both longitudinal and transverse division take place, as seen both in fresh and stained preparations. Probably there is a periodicity in the direction of division followed by the Spirochaetes of Lamellibranchs, just as there is a periodicity in the case of the blood-inhabiting Spirochaetes. Miss Porter and I (1909) have found that the Spirochaetes of relapsing fevers, *S. recurrentis* and *S. duttoni*, divide longitudinally at the beginning and end of infection when relatively few Spirochaetes are present in the blood, while they divide transversely during the height of infection when Spirochaetes are numerous in the blood. I have already recorded (VI. 1907, p. 499; I. 1908, pp. 42—46), that *S. balbianii* and *S. anodontae* divide both longitudinally and transversely. The technical difficulties in the way of examining portions of an infected Lamellibranch crystalline style from time to time are much greater than in examining drops of blood from a small mammal infected with Spirochaetes. For this reason it will be difficult to determine periodicity in the direction of division of the Spirochaetes of Lamellibranchs.

Longitudinal division of the Spirochaetes of *Tapes aureus* begins by the splitting (Pl. VI, Fig. 39) of the membrane lengthwise into two (Fig. 40). In such forms the two edges of the membrane are clearly distinguishable (Fig. 40). I have found several such Spirochaetes, with double membranes, in the same preparation, and not scattered at random through the various preparations made at different times. The probability of the occurrence of a periodicity in the direction of division of Spirochaetes in the Lamellibranchs is thus increased. Borrel (1908) has suggested that the presence of a double membrane in Spirochaetes stands in relation to their power of reversing their movements. However, all Spirochaetes do not possess double membranes; on the contrary, such forms are relatively few, and only occur at the onset of a period of longitudinal division, so that Borrel's interpretation cannot be accepted.

Some stages in the gradual separation of daughter forms of different individual Spirochaetes undergoing longitudinal division are shown in Pl. VI, Figs. 41—44.

Transverse division is brought about by constriction in the middle of long forms. Sometimes the constriction is a gradual one (Pl. VI, Fig. 52), accompanied by slight lengthening of the parent organism, and two daughter forms with pointed ends result (Fig. 17). At other times the constriction appears to be an abrupt one (Fig. 51) and daughter forms, with one end more or less rounded, result (Fig. 26). (Cf. Fantham, I. 1908, Pl. 3, Fig. 19, and Schellack, 1909, Pl. IV., Figs. 51, 55.)

No septum was seen in any case of transverse division of Spirochaetes examined.

The doubling of a Spirochaete on itself, as represented in Pl. VI, Figs. 45—47, is suggestive of division. However, I did not see division of the organism occur during my observations on living Spirochaetes in this condition (see p. 394).

Explanations of the Morphological Variation of the Spirochaetes of Tapes aureus.

(*a*) *The Hypothesis of Species Differences.* The hypothesis that the different forms belong to different species is unsatisfactory, because the examination of a very large number of Spirochaetes from many specimens of *Tapes aureus* shows that there are no separate or distinctive differences between the various forms. The Spirochaetes can be arranged in a continuous series, no matter what particular external feature of the parasite be used as a test. Where, then, in such a series can the limits for species be applied? It is obviously artificial to create barriers between forms that gradually merge into one another in nature.

(*b*) *The Hypothesis of Sexual Polymorphism.* That male, female and indifferent forms of Spirochaetes occur in *Tapes aureus* would doubtless be asserted by those who believe that sexual forms of Spirochaetes exist. "Male" individuals would be represented by the A forms (Text-fig. 2 A, Pl. VI, Figs. 1—5), "female" forms would be represented by the C forms (Text-fig. 2 C, Figs. 34—37), while "indifferent" forms would be found in group B. The great variation exhibited by the "indifferent" individuals would necessitate a reclassification of these forms, some probably being assigned the attributes of the "male" sex (Fig. 10), while others would probably be termed "female" (Figs. 29—32)—an unsatisfactory procedure. Such an important matter as the existence of sexual polymorphism in Spirochaetes cannot be decided by the examination of stained specimens alone. It is essential that it should be determined by the examination of the living organisms. Although I have examined many hundreds of living Spirochaetes from *Tapes aureus* I have never seen one that exhibited any sexual attributes, and until actual conjugation of the living organisms has been witnessed, I believe that it is useless to assign the attribute of sex to any form of Spirochaete. The hypothesis of sexuality, as explaining the morphological differences exhibited by the Spirochaetes of *Tapes*, is, in my opinion, quite untenable.

(*c*) *The Hypothesis of true Morphological Variation due to Division and Growth.* The various forms of Spirochaetes from *Tapes aureus* may be broadly classified as being long (Pl. VI, Figs. 5, 15, 18, 19, 20, 33), or short (Figs. 1, 8, 9, 11, 12, 34), narrow (Figs. 1—5), or broad (Figs. 35—37), or as possessing ends that are pointed (Figs. 17, 19, 20, 21), tapering (Figs. 15, 32, 35), or round (Figs. 14, 30, 34).

The *differences in length* of the Spirochaetes are explicable by the direction of division. Transverse division of a long thin individual (Pl. VI, Fig. 5) results in the formation of two short daughter forms (Figs. 1, 2), while somewhat broader forms (Figs. 15, 18) that divide transversely (Figs. 50—52) produce individuals of medium length (Figs. 14, 17), about half that of the parent. Growth in length of the daughter forms occurs producing individuals similar to those shown in Figs. 16, 21, 25. All gradations in length are explicable as being due to transverse division followed by the growth of the daughter organisms. Marked variations (50μ to 150μ) have also been recorded in the length of *S. balbianii* in the oyster by all workers who have studied that parasite.

Variations in breadth are produced by longitudinal division. Moderately broad individuals divide longitudinally (Pl. VI, Figs. 39, 40, 42) and the daughter forms are about half the breadth of their parents (Figs. 7, 8, 42). Very narrow forms also divide longitudinally (Figs. 43, 44) and give rise to fine, almost hair-like Spirochaetes (Figs. 1—3). Longitudinal division of thick forms produces parasites of medium breadth.

Variations in length and breadth of the Spirochaetes of *T. aureus* are due then to growth and division. Thin forms (type A)—resulting from the longitudinal division of broader forms—are capable of growth in length and thickness and become Spirochaetes of the B type (Pl. VI, Figs. 15, 18, 19). Transverse division occurs in the B forms (Figs. 50—52), and the daughter organisms, either directly or by growth in thickness, give rise to parasites of the C and D types (Figs. 28—37). It is among the D forms that longitudinal division is common (Figs. 39, 40).

Differences in the shape of the ends of the Spirochaetes examined are probably due, in part, to slight differences in the process of transverse division. Sometimes the parent forms seem to elongate slightly and then gradually to constrict in the middle (Pl. VI, Figs. 49, 50). The resultant daughter forms would possess tapering ends (Figs. 9, 16). Parasites with tapering ends resemble the *S. balbianii* figured by Certes (1882) from oysters at Arcachon and La Rochelle. In some specimens the dividing Spirochaete seems to constrict more suddenly without much increase in length (Fig. 51). The daughter individuals from such a division have one end slightly more rounded than the other (Fig. 26), the rounder end being the one formed at the point of fission of the parent. Again, very gradual elongation and constriction of the dividing Spirochaete may occur (Fig. 52), and the individuals resulting from division tend to have at least one end pointed (Fig. 17), namely, that end formed by the breaking across of the elongate, median constriction of the parent.

In view of the many differences of form directly attributable to the processes of growth and division in Spirochaetes, I conclude that true morphological variation is sufficient to account for any form of Spirochaete that has been observed in *Tapes aureus*. Consideration of the series of the figures set forth in Plate VI, justifies this conclusion, at any rate in the present state of knowledge.

Marked morphological variation is not unknown among the parasitic Protozoa. Good examples of such variation may be found in various species of *Crithidia* and *Herpetomonas*, so that the occurrence of morphological variation among Spirochaetes is not surprising.

The possible Infection of Tapes aureus with Spirochaeta balbianii directly from Oysters.

The specimens examined from the first batch of *Tapes aureus* received by me at Banyuls were found to be uninfected with Spirochaetes. Oysters were also obtained locally at Banyuls, and most of them were found to be infected with *S. balbianii* (Pl. VI, Fig. 38).

One of the *Tapes* from the first batch which was uninfected with Spirochaetes, so far as could be ascertained without injuring the living specimen, was placed in an aquarium of sea-water along with several living oysters containing *S. balbianii.* After a period of three weeks the *Tapes* was dissected and found to be infected with Spirochaetes, which were identical morphologically with *S. balbianii.* In all probability, then, the specimen of *Tapes* had become infected with *S. balbianii* derived from the oysters kept in the same aquarium. I have previously recorded (VI. 1907, p. 500; I. 1908, p. 10) the fact that *S. balbianii* was found swimming about freely in a basin of sea-water in which infected oysters were being kept alive. I think it is almost certain that Spirochaetes from the oyster had swum into the water of the aquarium and infected the *Tapes per os.* Further infection experiments of this nature are necessary. Rigorous proof of the conveyance of infection by the water can only be obtained, however, by breeding young *Tapes* from parents definitely known to be uninfected, in sea-water also known to be free from Spirochaetes, and then placing infected oysters with the *Tapes.* I hope to have an opportunity of investigating this problem in the future.

Oysters and *Tapes* may occur together naturally. The Spirochaetes found in *Tapes aureus* seem to me to be *Spirochaeta balbianii* or only varieties of the same. It is probable that *S. balbianii* occurs in many marine Lamellibranchs.

As corollaries to the foregoing observations I would suggest that it is not entirely wise to create many new species of Spirochaetes—as Schellack (1909) has done—because of their occurrence in different, yet often closely allied, marine Lamellibranchs, without first examining large numbers of Spirochaetes from each host. Such extended examination is necessary in order to acquire knowledge of the *morphological variation* of the Spirochaetes. Furthermore, infection experiments should also be considered.

CONCLUSIONS.

(1) The Spirochaetes of *Tapes aureus* are probably all referable to one species, for a continuous series can easily be found embracing long and short, narrow and broad forms, also Spirochaetes with sharply pointed, tapering or rounded ends and others whose two ends are unequally pointed or rounded (Pl. VI, Figs. 1—37).

(2) All the differences in morphology and in size of the Spirochaetes observed in *Tapes aureus* are explicable by reference to the processes of division and growth.

(3) The Spirochaete found in the crystalline style of *Tapes aureus* is probably either *S. balbianii* (which occurs also in the oyster) or a variety of the same.

(4) The creation of several different species of Spirochaetes in the same host is not to be commended until a careful study of the morphological variation of the Spirochaetes has been made.

(5) Infection experiments should be carried out before it is assumed that each species of Lamellibranch harbours its own particular species of Spirochaete. It is probable that *S. balbianii* is widely disseminated among various marine Lamellibranchs.

(6) Both longitudinal (Pl. VI, Figs. 39—44) and transverse division (Figs. 49—52) occur in the Spirochaetes of *Tapes aureus*, as in *S. balbianii* of the oyster, *S. anodontae*, and the blood-inhabiting Spirochaetes *S. recurrentis* and *S. duttoni*. It is possible that there is a periodicity with regard to the mode of division (transverse and longitudinal) in the Spirochaetes of Lamellibranchs, such as Miss Porter and I (1909) have found for *S. recurrentis* and *S. duttoni*.

(7) No evidence of sexual differentiation, conjugation or encystment was found while examining the Spirochaetes of *Tapes aureus*.

(8) The evidence at hand is insufficient to establish the existence of more than one species of Spirochaete in *Tapes aureus*.

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EXPLANATION OF PLATE VI.

All figures were outlined with an Abbé-Zeiss camera lucida, using a Zeiss 2 mm. apochromatic or $\frac{1}{12}$ inch achromatic objective and compensating ocular 8.

The magnification is, in all cases, except Fig. 54, approximately 1300 diameters.

Figs. 1—37 are arranged to show gradual increase in breadth of the various specimens of Spirochaetes found in *Tapes aureus.*

Figs. 1—5. Very thin forms of Spirochaetes from *Tapes aureus.* The edge of the membrane is well shown, but the organisms are too thin for the internal chromatin apparatus to be seen clearly, except in a few areas. These thin forms are of variable length, and result from longitudinal division. The Spirochaete drawn in Fig. 5 is probably about to divide transversely to produce a form similar to that shown in Fig. 1. (Iron-haematoxylin.)

Figs. 6, 7. Slightly thicker forms, with rounded ends and variable length, in which the transverse bars of chromatin could be well seen in parts. (Twort's stain.)

Figs. 8, 9. Short, somewhat thicker Spirochaetes, with relaxed membrane, showing myonemes. The ends of the organisms are tapering. (Delafield's haematoxylin.)

Fig. 10. Thin Spirochaete with pointed ends, showing clearly the transverse bars of chromatin. The membrane is probably very closely contracted round the body, and the edge is only seen at one point. (Iron-haematoxylin.)

Fig. 11. Small (young) Spirochaete with well-marked relaxed membrane showing myonemes. The membrane is slightly folded over at its upper end. The parasite occurred in a preparation in which there were many forms like those drawn in Figs. 29—32. (Delafield.)

Fig. 12. Spirochaete showing marked spiral coiling and slightly dissimilar ends. The edges of the membrane and body are not clearly distinguishable from each other. (Delafield.)

Fig. 13. Rather long, relatively straight form, with one end pointed, the other end rounded. Membrane contracted against the body. (Delafield.)

Fig. 14. Spirochaete with many coils and rounded ends. Internal chromatin apparatus not well shown. (Delafield.)

Fig. 15. Long Spirochaete, with coils somewhat flattened. The ends are tapering and membrane closely contracted. (Iron-haematoxylin.)

Fig. 16. Spirochaete with tapering ends. Internal structure not very clearly seen. (Delafield.)

Fig. 17. Shorter form with pointed ends. Produced by transverse division of such a form as shown in Fig. 15. (Iron-haematoxylin.) Note the variation in degree of the tapering of the ends in Figs. 13—17.

Fig. 18. Long Spirochaete with edge of membrane clearly stained lying close to or along the body. Ends somewhat rounded. (Iron-haematoxylin.)

Fig. 19. Long Spirochaete with pointed ends. Membrane not well-marked. The spiral character of the internal nuclear apparatus is seen near the upper end. (Iron-haematoxylin.)

Fig. 20. Long Spirochaete with pointed ends. The edge of the contracted membrane is seen at intervals. Some of the chromatin bars show signs of division at the upper end. (Iron-haematoxylin.) Many similar forms occurred in a preparation treated with gold-chloride.

Fig. 21. Spirochaete with pointed ends. (Iron-haematoxylin.)

Fig. 22. Spirochaete which is very like those drawn in Figs. 19—21, but possesses rounded ends. (Delafield.)

Fig. 23. Parasite with tapering ends and well-marked terminal basal granules. (Delafield.)

Fig. 24. Parasite with rounded ends, showing clearly the edge of the coiled membrane. After treatment with Twort's stain (neutral red and Lichtgrün) the edge of the membrane is stained red, with a delicate green film around it.

Fig. 25. Spirochaete with clearly marked internal structure and approximately rounded ends. (Delafield.)

Fig. 26. Spirochaete from the same preparation as that of Fig. 11, but slightly larger (older). Membrane relaxed, showing myonemes. (Delafield.)

Fig. 27. Long Spirochaete with rounded ends, slightly longer than that shown in Fig. 24, and from the same preparation. (Twort's stain.)

Fig. 28. Long Spirochaete with rounded ends and chromatin arranged internally as dots or granules, not bars. (Delafield's haematoxylin.) Similar forms are seen on preparations stained with Twort's stain.

Figs. 29—32. Typical Spirochaetes with undulatory outlines and membranes. These parasites were fairly broad. All from the same preparation. Lengths variable, and in some cases the ends were slightly tapering. (Delafield.)

Fig. 33. Long Spirochaete with lower end slightly tapering. (Delafield.)

Fig. 34. Shorter, broader parasite with rounded ends. Thionin. This Spirochaete from *Tapes aureus* is a typical short *S. balbianii* form, as seen in the oyster.

Figs. 35—37. Somewhat broad forms from *Tapes aureus*. Their outlines are thrown into a few undulations of relatively great amplitude. The membrane of each is closely contracted round the body, but the edge of the membrane is, in each case, deeply staining. The ends are tapering to a variable degree, those of the same parasite often tapering unequally. These broad forms were found in the same preparation, stained with iron-haematoxylin. They were not very numerous, for there was a fair number of forms like those drawn in Figs. 19—21, and a large number of thin forms (cf. Figs. 1—5). Similar parasites seen in a preparation stained with gentian violet.

Fig. 38, *S. balbianii* from an oyster obtained at Banyuls. The parasite is somewhat broad, and has distinctly rounded ends. Many of the Spirochaetes occurring in the same oyster were longer than the one drawn. (Delafield.)

Figs. 39—44 illustrate longitudinal division.

Fig. 39. Spirochaete whose membrane has divided at its lower end. The internal nuclear apparatus is arranged as a flattened spiral especially near the ends. Longitudinal division of the parasite similar to the one figured gives rise to forms like that drawn in Fig. 6. (Delafield.)

Fig. 40. Spirochaete with double membrane and two sets of myonemes, resulting from division. (Delafield.)

Fig. 41. Short thin form with membrane in process of longitudinal division. The parasite has already divided at the lower end. (Delafield.)

Fig. 42. Longitudinal dividing form. The daughter parasites are separate for most of their length, but remain still unsplit at the upper end. (Delafield.)

Fig. 43. Longitudinal division, the daughter forms having become free at their lower end. One of the daughter forms has its free end curled round. (Delafield.)

Fig. 44. Nearly completed longitudinal division of a relatively thin Spirochaete into two thinner forms like those shown in Figs. 1—5. (Iron-haematoxylin.)

Figs. 45—48. Bent or looped forms. In Fig. 46 the Spirochaete has bent in the middle and the two limbs intertwined. In Fig. 48 only one end has bent round. All stained with iron-haematoxylin, except Fig. 47, which was stained with Delafield's haematoxylin.

Figs. 49—52 illustrate transverve division.

Fig. 49. Parasite with slightly tapering ends about to divide. A slightly arched constriction appears at the centre, which is unstained internally. By its division the parent produces forms of the type shown in Fig. 16, though naturally shorter at first.

Fig. 50. Parasite with slightly constricted unstained area in the middle. Division takes place at the unstained area.

Fig. 51. Spirochaete about to divide at the abrupt constriction in the middle, producing daughter forms with more or less rounded ends, like that shown in Fig. 26.

Fig. 52. Spirochaete with very tenuous centre, just about to produce individuals with pointed ends, like that shown in Fig. 17.

Fig. 53. Parasite drawn to show the extreme flexibility of Spirochaetes. This form, originally resembling that drawn in Fig. 15, has become coiled in a most complicated manner at one end, resulting in the production of a "pseudo-cyst."

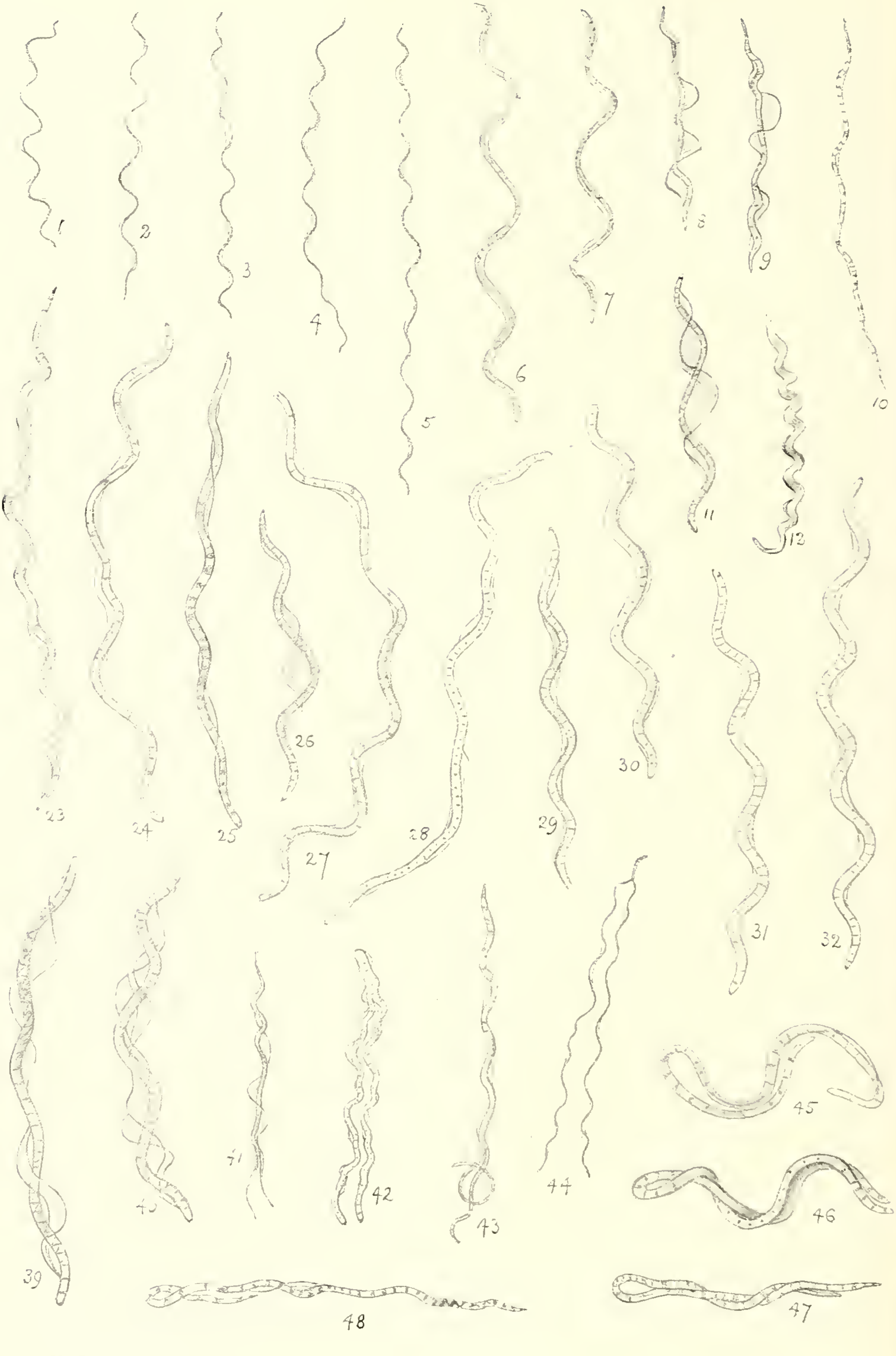
Fig. 54. Transverse sections of Spirochaetes from serial sections of an infected crystalline style of *Tapes aureus*. × 1750 approximately.

a. Section of Spirochaete passing between the chromatin bars. Membrane contracted close to the body, and seen as a granular projection on the right of the body.

b. Section of larger Spirochaete, passing through chromatin bar. Membrane seen as a lateral extension of the periplast.

c. Section of Spirochaete, passing through edge of chromatin bar. Membrane shown as periplast extension.

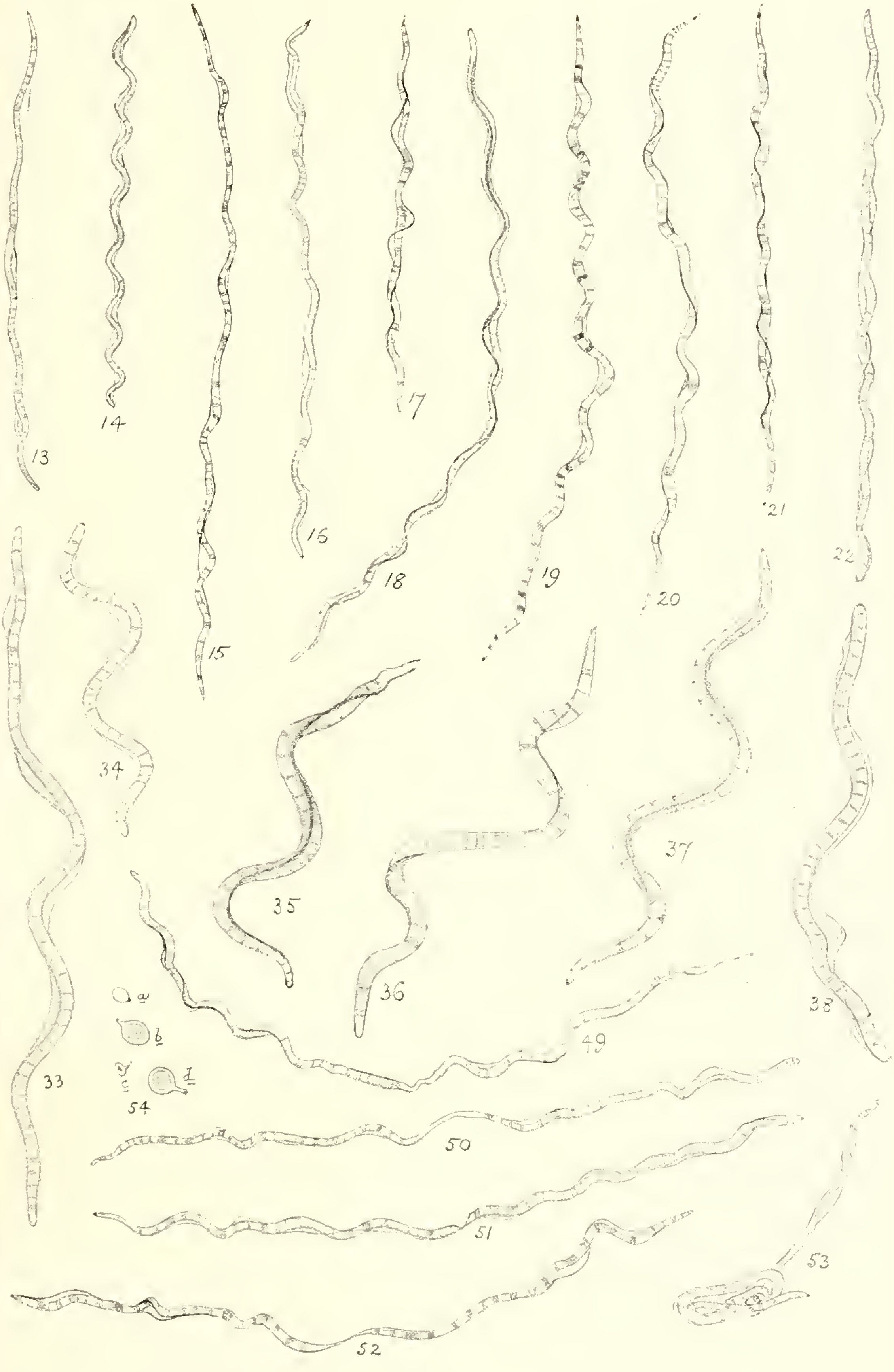
d. Section of broad Spirochaete through bar of chromatin. Thickened chromatic edge of membrane seen.



H. B. Fantham, del.

THE SPIROCHAETES

PLATE VI



OF TAPES AUREUS.

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NATURAL HISTORY

THE DRUG TREATMENT OF CANINE PIROPLASMOSIS.

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1. Some Earlier Work by Italian Investigators.

Before describing my further results in the treatment of canine piroplasmosis by aniline dyes, I desire to mention some earlier experiments, carried out by Italian investigators whose papers have until recently escaped my attention. The results of the Italian experiments are cited in considerable detail with a view to making it unnecessary for other workers to refer to the original papers which may be difficult of access. It is well to note that the authors in question give very little information concerning their experiments; thus Memmo, Martoglio and Adani are satisfied with giving mere clinical notes, whilst Levi della Vida omits a number of essential data from his protocols.

Attempts to cure the disease by artificially induced Leucocytosis: treatment with Quinine, Oil of Turpentine, Sodium nucleinate.

Memmo, Martoglio and Adani (1905, p. 23), who observed canine piroplasmosis at Cassala in Eritrea, state that before the nature of the disease was recognised in that part of Africa "anaemia of dogs" used to be treated with antihelminthics and by subcutaneous injections of ferruginous preparations. They treated two dogs by subcutaneous injections of *quinine* and the animals recovered, abscesses having formed at some of the places where the injections had been made. They cite the experience of Mozzetti, who stated that he had found recovery to take place more readily in dogs in whom abscesses had developed following upon subcutaneous injections of drugs. Memmo, Martoglio and Adani thereupon decided to try if the *artificial production of abscesses* in dogs would exert an influence upon the course of the disease. They appear to have had striking results, for 12 dogs with piroplasmosis in which abscesses were produced all recovered. The dog suffering from piroplasmosis received 5 to 10 drops of pure *oil of turpentine* injected subcutaneously in the pectoral region. The injection produced a violent local reaction and considerable fever. The abscess which developed at the seat of injection was lanced after 2—3 days, after which the temperature fell rapidly, the animal's condition improved, the blood again became normal and the dog recovered completely. They proposed to try the treatment on cattle.

Our exact experiments (Nuttall and Graham-Smith, 1908, p. 121) have demonstrated that quinine is ineffective in the treatment of the disease and those of Levi della Vida (1907, p. 359), with turpentine, gave results contrary to Memmo, Martoglio and Adani's. Levi della Vida inoculated 3 dogs with piroplasmosis, and after 3 to 4 days he gave each dog a subcutaneous injection of ·5 c.c. of oil of turpentine. The dogs had marked leucocytosis (12,000 to 15,000 per c.mm.) following upon the turpentine injections, but they all died of piroplasmosis within the usual time, 5 to 8 days, after inoculation.

Levi della Vida likewise induced leucocytosis in two dogs through intraperitoneal injections of *sodium nucleinate* (3 c.c. of a 3 % solution). The results were negative and similar to those obtained with turpentine.

Attempts to cure canine piroplasmosis by means of Atoxyl, Fowler's solution, Sodium cacodylate, Methylene blue, Brilliant green, Trypanred, and Dichlorobenzidine + 2 mol. of Amido-naphthol-disulpho.

Levi della Vida (1907, p. 359), working with a strain of canine piroplasmosis obtained from Hamburg, attempted to cure the disease by means of various drugs as follows:

Atoxyl. The results obtained with atoxyl treatment are contrary to those of Gonder (1908) which we have fully described elsewhere (This *Journal,* Vol. I. p. 223). Levi della Vida (p. 360) does not mention the number of experiments he performed on dogs, whereas Gonder gives full details of six cases treated with negative results. Levi della Vida states that he gave his dogs 0·20 centigrammes of atoxyl every day, the treatment commencing 48 hours after inoculation. Parasites never appeared in the atoxyl-treated dogs, whereas untreated control dogs died of piroplasmosis. The atoxyl-treated dogs subsequently proved to be susceptible to piroplasmosis when reinoculated. One dog, which received less atoxyl than the amount stated, was not cured. This is all that the Italian author has to say about his experiments, consequently little value can be attached to them, especially since they are contrary to the carefully conducted experiments reported by Gonder. There is no evidence that atoxyl exerts any curative effect upon the disease when clinical symptoms have developed.

Two dogs were similarly treated with *Trypanred* (0·25 gramme), the one after the parasites had appeared in the blood, the other at the time when it was inoculated; this dye likewise exerted no apparent effect upon the course of the disease. (This result is contrary to those obtained by Nuttall and Hadwen, VI. 1909, p. 172, in which a dog was cured by means of this dye, and the degeneration of the parasites was observed in two dogs in consequence of the treatment.)

Levi della Vida (p. 360) records that *Dichlorobenzidine* + 2 molecules of *Amido-naphthol-disulpho* (Friedr. Bayer and Co., Elberfeld) may at times exert a curative action. In two dogs which received 0·16 gramme of this dye daily on four successive days, beginning with the day when parasites appeared, the disease was promptly arrested; the parasites grew scarcer and finally disappeared. The blood of these dogs was found to be infective after one and two months had elapsed. Whereas one dog showed a fall in the number of red blood-corpuscles the contrary was the case in the other animal; both showed leucocytosis (10,000 per c.mm.); neither of them had jaundice or haemoglobinuria, whilst in both the temperature fell to normal after the first few days. Both of these dogs were moderately young. When the treatment was tried on 8 puppies, 2 to 3 months old, all of the animals died of piroplasmosis. The dye was given in doses of 10 to 15 centigrammes per day, treatment commencing at different times following upon inoculation. The dye exerted a parasiticidal effect *in vitro,* for virulent blood exposed for 15 to 30 minutes to the action of a 5 % solution of the dye proved to be non-infective when injected into two dogs.

Fowler's solution. Levi della Vida states that he treated one dog with ·01 to 0·15 gramme of Fowler's solution per day. A few parasites appeared in its blood and the disease ran a mild course. This result may very well be attributed to the mild strain of piroplasmosis with which the author was working. In the absence of careful observations upon the parasites, and in the absence of control experiments, no conclusions can be reached regarding the value of this mode of treatment.

Sodium cacodylate. Levi della Vida states that he treated 7 dogs with this drug and that only one died of piroplasmosis. In the 6 dogs which recovered the drug was given in doses of 15 to 20 centigrammes per day during 4 to 6 days. In 5 dogs treatment began 48 hours after inoculation and in 1 dog 4 days after inoculation. Of the 6 dogs 3 never showed parasites, whereas the other 3 suffered from a mild form of the disease. The three dogs in which parasites did not appear subsequently proved to be susceptible to infection through the agency of ticks (no particulars given). Blood mixed with a 1·8 % solution of the drug in ·9 % NaCl solution and allowed to stand 2 hours at room temperature remained infective. In the absence of further details regarding these experiments, the most that can be said is that it appears expedient to repeat them. I propose to do this, and shall report at a later date upon the results.

Experiments with various dyes. Levi della Vida (1907, p. 359) found that *Methylene blue* exerted no effect upon the course of the disease in two dogs which received 4 centigrammes of the dye injected subcutaneously as soon as the parasites appeared in the animals' blood. (These results accord with those of Nuttall and Graham-Smith, 1908, p. 222.)

Brilliant green injected subcutaneously at the same time as the inoculation was made did not lengthen the period of incubation nor retard the death of a dog thus treated, the dose administered amounting to ½ c.c. of a 0·5 % solution of the dye. A second dog received two subcutaneous injections, each of 1 c.c. of a 0·5 % solution of brilliant green, after the parasites had appeared in its blood, but the dye exerted no apparent effect; the dogs died of piroplasmosis on the 9th day. (These results accord with those obtained in my experiment recorded below and carried out before I was aware of the experiments of Levi della Vida.)

CONCLUSIONS REGARDING THE ITALIAN EXPERIMENTS.

None of the dyes tested by Levi della Vida could, therefore, in the light of his experiments, be regarded as of value in the treatment of canine piroplasmosis. The results obtained by this author and Memmo, Martoglio and Adani with other modes of treatment are either contradictory or doubtful. The experiments with sodium cacodylate require to be repeated.

2. **Further Experiments with Dyes.**

The following protocols relate to experiments which I have carried out with Brilliant Green, Benzopurpurine and Congo Red:

EXPERIMENT WITH BRILLIANT GREEN.

Dog 1: Fox-terrier puppy. Weight 9 lbs.

Received one subcutaneous injection of Brilliant Green in 1 % watery solution 24 hours after the parasites first appeared in the dog's blood[1].

Date	Day	Temp.°F.		% of infected r.b.c.	%								
					(P)	(O)	(PP)	(D)	(P$_4$)	(PPO)	(OO)	(O$_4$)	(P$_6$)
17. VII. 09.	1	—	3 p.m. *Inoculated* with 4 c.c. of virulent heart-blood.										
	2	—											
	3	103·4											
	4	102·8											
	5	102·6	*Parasites appeared:* very few at film-edge: 3 (PP), 1 (O).										
	6	103·0	10.30 a.m. more parasites	0·1	2	40	47	2	9	·	·	·	·
			11 a.m. *Treatment:* 4·5 c.c. Brilliant Green sol.										
			3 p.m. ,, ,, ,, ,,	0·2	3	34	60	2	1	·	·	·	·
			5 p.m. ,, ,, ,, ,,	3·0	3	45	38	2	2	·	·	·	·
		102·8	5.30 p.m. Swelling at site of drug-injection.										
	7	—	— a.m. ,, ,, ,, ,,	0·5	5	39	53	1	2	·	·	·	·
		103·4	12 m. Swelling lanced.										
		104·0	5 p.m.										
	8	104·2	— a.m.	4·0	5	44	46	3	2	·	·	·	·
25. VII. 09.	9	104·2	10.30 a.m. *Dog moribund;* haemoglobinuria, blood watery	30·0	4	38	39	4	7	1	4	1	2
			11 a.m. Dog killed. The usual appearances of piroplasmosis were observed at autopsy. Very little colour was left at the seat of the dye injection.										

Control Dog: see under the following experiment.

---

[1] The abbreviations in this paper are similar to those used in our previous publications. (For details of methods used, see p. 161, this volume):

The signs		Stand for corpuscle containing
(P), (PP), (P$_4$), (P$_6$)	...	1, 2, and 4 or more pyriform parasites respectively.
(O), (OO) ...	...	1 and 2 rounded parasites respectively.
(PPO) ...	...	2 pyriform and 1 rounded parasite.
(D), (DD) ...	...	1 and 2 dividing forms respectively.

The abbreviation r.b.c. stands for red blood-corpuscles.

The + signs indicate that parasites were present, but that the percentage of infected r.b.c. was too small to determine without counting many thousands of corpuscles.

EXPERIMENT WITH BENZOPURPURINE.

Dog 2: Fox-terrier puppy. Weight 9¼ lbs.

Received one subcutaneous injection of Benzopurpurine in 1 % watery solution, 48 hours after the parasites first appeared in the dog's blood.

Date	Day	Temp.°F.		% of infected r.b.c.	%						
					(P)	(O)	(PP)	(D)	(P_4)	(PPO)	(OO)
17. VII. 09.	1	—	3 p.m. *Inoculated* with 4 c.c. of virulent heart-blood.								
	2	—									
	3	103·2	a.m.								
	4	103·6	a.m.								
	5	103·4	a.m. *Parasites appeared:* very few, 3 (PP) found.								
	6	103·0	a.m. Still very few parasites at film-edge: 3 (PP), 2 (P_4).								
	7	104·0	12 m. ,, ,, ,, ,, ,, ,,	1·2	1	48	45	0	3	0	0
			12.30 p.m. *Treatment:* 4·5 c.c. Benzopurpurine sol.								
			3 p.m.	1·6	5	26	61	2	5	0	1
		103·6	5 p.m.								
	8	103·4	— a.m. Haemoglobinuria, blood watery	8·0	4	42	43	1	4	5	1
25. VII. 09.	9	—	— a.m. *Dog found dead*, with the usual appearances due to piroplasmosis; blood watery, etc. Seat of dye injection slightly stained.								

Control Dog 1. Spaniel puppy. Weight 27 lbs.

17. VII. 09.	1	—	3 p.m. *Inoculated* with 4 c.c. of virulent heart-blood.
	2	—	
	3	104·0	
	4	104·8	
	5	104·4	a.m. *Parasites appeared:* only one infected r.b.c. found: 1 (PP).
	6	103·6	a.m. Very few parasites: 4 (PP) found at film-edge.
	7	105·0	a.m. Not examined.
	8	104·2	a.m. One infected r.b.c. per field.
25. VII. 09.	9	—	10 a.m. *Dog found dead*, with usual appearances of piroplasmosis; haemoglobinuria, etc.

CONCLUSIONS REGARDING THE FOREGOING EXPERIMENTS WITH BRILLIANT GREEN AND BENZOPURPURINE.

Reference to the percentage counts of the different types of parasites encountered in the treated dogs' corpuscles renders it clear that Brilliant Green and Benzopurpurine exert no injurious effect upon the parasites and that the piroplasms multiply in the usual manner up to the time of the animals' death. Judging from the few figures contained in the protocols it would appear almost as if the injection of the dyes increased the percentage of (PP) parasites, thus:

In Dog 1 the (PP) parasites numbered 47 % before, 60 % 4 hours after treatment.
In Dog 2 ,, ,, ,, ,, 45 % ,, 61 % 2½ ,, ,, ,,

EXPERIMENTS WITH CONGO RED.

Dog 3: Fox-terrier, aged about 1 year. Weight 14 lbs.

Received one subcutaneous injection of 4 c.c. of 1 % watery solution of Congo Red.

Date	Day	Temp.°F.	
16. x. 09.	1	—	*Inoculated* with 5 c.c. of virulent heart-blood (from Dog 48).
	2	—	
	3	102·0	
	4	103·0	
	5	105·2	
	6	104·0	
	7	106·0	10 a.m. *Parasites appeared:* 3 (O), 4 (PP). 11.50 a.m. *Treatment* with Congo Red.
	8	103·6	No parasites found.
	9	102·6	,, ,,
	10	102·6	,, ,,
	11	103·2	,, ,,
	12	104·0	,, ,,
	13	105·0	,, ,,
	14	104·6	,, ,, Dog very weak.
	15	104·0	,, ,, ,, ,,
31. x. 09.	16	95·0	a.m. No parasites found. Dog very weak. 7 p.m. *Dog found dead.* Smears from heart, spleen and liver showed no parasites. Death probably due to the drug which apparently destroyed both the dog and the parasites.

Dog 4: Terrier mongrel, aged about ½ year. Weight 9 lbs.

Received one subcutaneous injection of 4 c.c. of Congo Red in 0·5 % watery solution 48 hours after the parasites appeared in the dog's blood.

250 r.b.c. counted for % infected, except on Day 14 when 500 were counted.

100 parasitized r.b.c. counted for types, except on Day 13 when 50 were counted.

Date	Day	Temp.°F.		% of infected r.b.c.	%							
					(P)	(O)	(PP)	(D)	(P_4)	(OO)	(O_{3-4})	(PPO)
26. x. 09.	1	—	*Inoculated* with 5 c.c. of virulent heart-blood (from Dog 2).									
	2	101·2										
	3	102·0										
	4	101·0										
	5	101·4										
	6	101·8										
	7	102·2										
	8	102·4	*Parasites appeared:* very few at film-edge: 3 (PP), 1 (O).									
	9	103·0	Few parasites at film-edge: 8 (PP), 6 (O).									
	10	104·8	— a.m.									
			12.15 p.m. *Treatment* with Congo Red. Haemoglobinuria ...	4·4	·	54	42	2	2	·	·	·
			2.15 p.m.	8·0	·	55	38	1*	3	3	·	·
		105·4	4.15 p.m. Chromatin of parasites tending to form one mass ...	13·6	·	51	43	·	2	4	·	·

* 1 (DD).

Date	Day	Temp. °F.		% of infected r.b.c.	%							
					(P)	(O)	(PP)	(D)	(P_4)	(OO)	$(O_{3\text{-}4})$	(PPO)
	11	103·6	9.40 a.m. Parasites appear small, ragged; chromatin in one mass	32·4	1	61	19	·	1	12	4	2
		103·0	5.10 p.m. Parasites stain badly, faint blue, r.b.c. crumpled	19·2	·	74	8	·	·	16	1	1
	12	101·4	9 a.m. Blood thinner, only doubtful pyriforms noted ...	14·4	·	75	8	·	·	15	2	·
		101·6	5.40 p.m. Blood very watery, r.b.c. stain faintly, parasites only show chromatin and little or no blue ...	7·2	·	69	1	·	·	29	1	·
	13	102·0	— a.m. Blood still more watery; all parasites ragged and faint	3·6	·	86	·	·	·	14	·	·
	14	99·4	10.30 a.m. Parasites; only chromatin ...	·4								
		100·8	5.30 p.m. Very few parasites; rounded, show little blue and stain faintly ...	+								
	15	101·0	9.30 a.m. Blood condition improving ...	0								
		101·2	— p.m. Urine normal.									
	16	104·2	9.30 a.m.	0								
		103·6	— p.m.									
	17	101·0	9.30 a.m. Blood condition improving ...	0								
		101·6	— p.m.									
	18	100·6	... ...	0								
		101·2										
	19	100·6	... ...	0								
	20	101·4	Dog lively ...	0								
	21	102·2	,, ,, ...	0								
	22	101·0	,, ,, ...	0								
	23	101·2	,, ,, ...	0								
	24	101·6	,, ,, ...	0								
	25	101·2	,, ,, Parasites reappeared: 3 (O), 2 (OO) at film-edge ...	+								

Date	Day	Temp. °F.		% of infected r.b.c.	%				
					(P)	(O)	(PP)	(D)	(OO)
	26	100·2	Dog lively. Parasites: 3 (O) at film-edge ...	+					
	27	100·4	Dog lively. Parasites:	·2	·	+	+	·	+
	28	103·6	,, ,, blood watery. Parasites: ...	2·0	·	85	15	·	·

23. xi. 09. 29 — 9 a.m. *Dog found dead.* Haemoglobinuria. Smears from various organs were prepared:

Kidney showed 15 % r.b.c. infected (PP), (PPPP), (O), etc.
Spleen ,, 15 % ,, ,, ,, ,,
Liver ,, 7·5 % ,, ,, ,, ,,
Heart about 6 %, blood clotted, roulleaux, very watery.

Control Dogs: Nos. 2 and 3. See p. 418.

Dog 5: Fox-terrier, aged about ½ year. Weight 8½ lbs.

Received one subcutaneous injection of 3 c.c. of Congo Red in ·5 % watery solution 24 hours after the parasites appeared. Counts of r.b.c. etc. as under Dog 4, *q.v.*

Date	Day	Temp. °F.		% of infected r.b.c.	%						
					(P)	(O)	(PP)	(D)	(P_4)	(OO)	(O_{3-4})
26. x. 09.	1	—	*Inoculated* with 5 c.c. of virulent heart-blood from Dog 2.								
	2	102·0									
	3	103·0									
	4	102·0									
	5	102·6									
	6	102·6									
	7	103·2									
	8	105·4	— p.m. *Parasites appeared*, few.								
	9	103·6	11.40 a.m. *Treatment* with Congo Red	6·4	2	45	49	1	3	·	·
			12.40 p.m.	8·0	1	39	55	·	5	·	·
			2.40 p.m. Haemoglobinuria	8·4	·	45	51	1	·	3	·
		105·2	5.40 p.m. R.b.c. crumpled, many (PP) appear degenerated	10·8	1	54	39	·	·	5	1
	10	104·2	9.40 a.m. Blood watery, (PP) as before	12·8	·	75	19	·	·	6	·
		102·0	4.40 p.m. (PP) doubtful, (O)s very small ...	11·6	·	86	3	·	·	11	·
	11	101·2	9.40 a.m. Urine lighter, blood watery, only chromatin of parasites with little or no blue seen in stained films	2·0	·	77	·	·	·	21	2
		100·4	5.40 p.m.	2·4	·	80	·	·	·	20	·
	12	99·4	9.30 a.m. Blood condition improving, less than ·1 % r.b.c. infected, parasites: chromatin only ...	+							
		99·2	5.40 p.m.	0							
	13	101·4	10 a.m. Blood watery ...	0							
	14	97·6	10.30 a.m. ,, ,, ...	0							
		99·0	— p.m. ,, ,, ...	0							
9. xi. 09.	15	95·0	— a.m. Blood very watery, dog weak	0							

2 p.m. *Dog died.* Organs anaemic. Very few parasites found in smears from heart, liver, kidney and spleen.

Control Dog 2: Fox-terrier, about ½ year old. Weight 8½ lbs.

Date	Day	Temp. °F.	
26. x. 09.	1	—	*Inoculated* with 5 c.c. of virulent heart-blood (from Dog 2).
	2	101·4	
	3	103·0	
	4	101·2	
	5	101·4	
	6	102·6	
	7	103·4	
	8	105·4	*Parasites appeared*, few.
	9	105·2	,, few.
	10	103·0	,, ,,
	11	103·8	,, ,,
	12	105·4	,, more. Haemoglobinuria.
7. xi. 09.	13	103·0	*Dog died of piroplasmosis.*

Control Dog 3: Collie pup, about ½ year old.

Date	Day	Temp. °F.	
26. x. 09.	1	—	*Inoculated* with 5 c.c. of virulent heart-blood (from Dog 2).
	2	103·2	
	3	103·8	
	4	103·0	
	5	103·8	
	6	103·6	
	7	102·6	
	8	102·4	*Parasites appeared:* only 1 (PP) found.
	9	102·4	,, increasing.
	10	103·0	,, 3 per field.
	11	104·8	,, numerous. Haemoglobinuria. Dog weak.
6. xi. 09.	12	—	9.15 a.m. Parasites numerous. *Dog died of piroplasmosis.*

CONCLUSIONS REGARDING THE FOREGOING EXPERIMENTS WITH CONGO RED.

The three experiments with Congo Red demonstrate very clearly that the dye exerts a direct effect upon the parasites. Unfortunately, the dye also appears to exert an injurious effect upon the dogs, for all of them died. The treated dogs' blood corpuscles appear to suffer, for many of them present a crumpled appearance.

In the case of **Dog 3** the dye-injection was given on the 7th day after inoculation, a few parasites having appeared on that day in the dog's blood. No parasites were discoverable in blood-films prepared on the 8th to 16th day, and no parasites could be detected in the dog's internal organs at autopsy.

In the case of **Dog 4**, the dye-injection was given 48 hours after the parasites appeared (10th day after inoculation). The percentage of infected corpuscles continued, however, to rise rapidly, so that the enumeration on the 11th day showed that 32·4 % (morning) and 19·2 % (afternoon) of the r. b. c. were infected. Nevertheless, the number of (PP) forms had fallen to 19 % and 8 % respectively. On the 12th day the percentage of infected r.b.c. continued to fall, and only 1 % of (PP) forms were encountered in the afternoon. On the 13th day only rounded parasites were encountered. On the 15th to 24th days no parasites could be detected, after which they reappeared and the dog died of piroplasmosis, the percentage of infected r.b.c. in the peripheral circulation being low (2 %), whilst high counts (6 to 15 %) were obtained from smears of the internal organs.

In **Dog 5** the parasites appeared on the 8th day after inoculation and the dye was injected on the 9th day. Here the percentage of infected r.b.c. rose very slightly on the 10th day, whilst the number of (PP) forms decreased to 19 % and 3 % in the morning and afternoon enumerations. On the 11th day only rounded parasites were encountered and no parasites were found after the afternoon of the 12th day. At autopsy, on the 15th day, only a very few parasites could be detected in the dog's internal organs.

N.B. It is necessary to repeat, in this connection, that in dogs which recover normally from piroplasmosis the behaviour of the parasites is quite different to that observed in dogs treated with trypanblue, trypanred, or Congo Red. Whereas, in *normal* recovery, whilst the percentage of infected corpuscles gradually decreases, *the pyriform parasites persist* as long as parasites are discoverable microscopically. On the other hand, after effective dye treatment, the percentage of infected corpuscles falls rapidly and *the pyriform parasites disappear.*

It is essential, therefore, when experimenting upon the possibly curative effect of such remedies to make accurate counts of the different types of parasites found in the peripheral blood of the experimental animals both before and after treatment.

3. The Continued Infectivity of the blood of dogs which have recovered from Piroplasmosis after Treatment with Trypanblue and Trypanred.

In a previous paper (Nuttall and Hadwen, VI. 1909; this volume, pp. 172, 176, 177, 180) the cases of 4 dogs were described which had survived an attack of piroplasmosis consequent upon treatment. The dogs (Nos. 1, 3, 4 and 6) appeared quite well at the time we published the account of our experiments. Whereas some of our other dogs, which had been successfully treated, succumbed to distemper, the four referred to escaped this intercurrent disease or (Dog 6) recovered from it. The following data refer to these four dogs whose previous history is in each case briefly summarized so as to render the subsequent record more intelligible.

Except for Dog 4, which is showing increasing signs of advancing age, all of the animals are at present healthy as far as outward appearances are concerned[1]. The body temperature has remained normal in the cases of Dogs 1 and 3 and in the cases of Dogs 4 and 6 has shown occasional slight rises which may have been due to other causes. The body-weight has increased in all cases: from 15 to 17 lbs. (232nd day, Dog 1), from 17½ to 21 lbs. (235th day, Dog 3), from 20 to 25 lbs. (227th day, Old Dog 4), and from 16 to 20 lbs. (210th day, Dog 6). Parasites have not again been detected in the peripheral blood upon microscopic examination.

On the other hand, the parasites must have persisted in the animals, for blood taken from the ear-veins of the four dogs gave rise to fatal piroplasmosis in all of the test dogs which were inoculated with it. Thus:

The blood of Treated Dog No.	Day after inoculation of Treated Dog when its blood was collected	No. of Test Dog inoculated	No. of days after inoculation when Test Dog died of piroplasmosis
1	143	I	19
3	190	II	16
4	182	III	13
6	165	IV	21
6	210	V	34

It is obvious from the foregoing that the treated dogs behave, in respect to the survival of the parasites in their blood, similarly to animals which recover under natural conditions. They represent "salted" animals in which parasites persist for an indefinite time without apparently producing any ill effects, the number of parasites present in the blood being exceedingly small. Although the test dogs which were inoculated with the blood of the recovered animals lived somewhat longer than is usual after inoculation with the blood from acute cases of piroplasmosis, there is no evidence that the parasites in the blood of the recovered animals had in any way become attenuated.

Two of the recovered dogs were, moreover, reinoculated with virulent blood, but neither of them showed any reaction. Dog 3 was reinoculated on the 221st day after the first inoculation, and Dog 4 was similarly reinoculated on the 213th day.

[1] Dog 6 has died since this was written, see protocol p. 422. It was killed by fighting with another dog on the 236th day after inoculation.

PROTOCOLS RELATING TO TREATED DOGS 1, 3, 4, AND 6.

Dog 1 (p. 172, this volume): Fox-terrier, about 2 years old. Weight 15 lbs. *Inoculated*, 1. III. 1909, with virulent blood. Parasites appeared in its blood on the 5th day. *Treatment:* Trypanred solution was injected four times on days 5, 7, 9 and 16. The parasites disappeared on the 15th day and reappeared in small numbers on the 29th day. A few parasites could be detected at the edge of blood-films up to the 85th day, after which they could no longer be found microscopically. The dog appeared healthy and well on the 111th day (17. VI. 1909) as recorded on p. 173. Weight on 101st day = 15½ lbs.

The following may be added to the published record referred to:

Date	Day	Temp. °F.	
18. VI. 09.	112–142	Normal.	
	143	,,	No parasites found. *Blood virulent:* fatal to Test Dog I, *q.v.*
	144–231	,,	
15. XI. 09.	232	,,	No parasites. Weight 17 lbs.
	233–280	,,	Ceased taking temperatures.
3. I. 10.	281	,,	Dog continues to look well. Weight 17¼ lbs.

Control Dog (No. II, p. 170) died on the 9th day after inoculation.

Dog 3 (p. 176, this volume): Adult Irish terrier. Weight 17½ lbs. *Inoculated*, 20. III. 09, with virulent blood. Parasites appeared 6th day. *Treatment:* Trypanblue solution was injected on the 7th day. The parasites disappeared on the 8th day from microscopic observation, a few parasites reappeared at the edges of blood-films on days 19, 21–23, 51–54, after which they were no longer found. On the 32nd day the dog weighed 17¾ lbs., on the 80th day 17¼ lbs. The dog remained lively and well up to the 90th day (17. VI. 09) as recorded on p. 176.

The record of this dog since the 90th day is as follows:

Date	Day	Temp. °F.	
18. VI. 09.	91–189	Normal.	
25. IX. 09.	190	,,	No parasites found. *Blood virulent:* fatal to Test Dog II, *q.v.*
	191–220	,,	
26. X. 09.	221	,,	*Inoculated* with 5 c.c. of virulent heart-blood from Dog 2 dead of piroplasmosis. (A control dog was inoculated at the same time with fatal results.)
	222–234	,,	
9. XI. 09.	235		Ceased taking temperature. Weight 21 lbs.
	236–289	,,	No temperature taken.
3. I. 10.	290	,,	Dog continues lively and well. Weight 17½ lbs.

Control Dog (No. IV, p. 170) died on the 7th day after inoculation.

Dog 4 (p. 177, this volume): Old Fox-terrier. Weight 20 lbs. *Inoculated*, 27. III. 1909, with virulent blood. Parasites appeared in its blood on the 6th day. *Treatment:* Trypanblue solution was injected on the 7th day. The parasites disappeared on the 9th day from microscopic observation; they reappeared in small numbers on the 19th day, and occurred occasionally in very small numbers at the edges of blood-films up to the 48th day, after which they could no longer be found microscopically. The dog appeared well and lively on the 83rd day (17. VII. 09) as recorded on p. 178 *q.v.*

The record of this dog since the appearance of our previous paper has been very satisfactory but for intercurrent mange.

Date	Day	Temp. °F.	
18. VI. 09.	84–88	—	Dog very well. Temp. normal. Weight on 86th day 23¾ lbs.
	89	104·0	
	90–93	Normal	
	94	103·6	Developed mange which became generalized and severe.
	95	103·4	
	96	103·2	
	97	102·4	
	98	103·2	
	99	102·4	
	100	103·2	
	101–181	Normal	
25. IX. 09.	182	,,	No parasites found. *Blood virulent:* fatal to Test Dog III, *q.v.*
	183–204	,,	
	205	103·4	Dog getting blind of one eye; old age.
	206–212	Normal.	
	213	,,	*Inoculated* with heart-blood of Dog 2, dead of piroplasmosis (control dog inoculated at the same time died of piroplasmosis).
	214	,,	
	215	103·0	
	216–226	Normal	
9. XI. 09	227	,,	Weight 25 lbs. Dog well since 84th day.
	228–281		Ceased taking temperature. Mange generalized.
3. I. 10.	282	,,	Dog continues lively. Weight 25¼ lbs.

Control Dog (No. VIII, p. 171) died on the 7th day after inoculation.

Dog 6 (p. 180, this volume): Young mongrel terrier bitch. Weight 16 lbs. *Inoculated,* 15. IV. 1909, with virulent blood. Parasites appeared in its blood on the 6th day. *Treatment:* Trypanblue solution was injected on the same day. The parasites disappeared on the 8th day, a few only being found at the edges of blood-films on days 18, 19, 35, 38 and 49. Showed symptoms of distemper on the 55th day, but recovered. Weight 14¾ lbs. on the 35th day. Dog looked lively and well and had no fever on the 44th to 65th day (17. VI. 09). Since then I have to record the following:

Date	Day	Temp. °F.	
18. VI. 09.	66	103·4	
	67–80	Normal	
	81	103·2	
	82	103·4	
	83–86	Normal	
	87	103·4	
	88–164	Normal	
25. IX. 09.	165	,,	No parasites found. *Blood virulent:* fatal to Test Dog IV, *q.v.*
	166–209	,,	
9. XI. 09.	210	,,	Weight 20 lbs. *Blood virulent:* fatal to Test Dog V, *q.v.*
	211–235	,,	No temperatures taken. Dog lively and well.
5. XII. 09.	236	,,	Killed by fighting with a terrier bitch. At autopsy no trace of blue colouration noticeable, no parasites found except for a very few large rounded intracorpuscular bodies with large central chromatin mass which were only encountered in spleen smears and will be described on a future occasion.

Control Dogs (Nos. VI and VII, p. 171) died respectively on the 8th and 36th days after inoculation.

PROTOCOLS RELATING TO TEST DOGS NOS. I TO V WHICH WERE INOCULATED WITH THE BLOOD OF THE RECOVERED DOGS.

Test Dog I. Collie, about ½ year old. Inoculated with blood from the ear-vein of Trypanred-treated Dog 1 on the 143rd day after the latter was inoculated.

Date	Day	Temp.°F.		% infected r.b.c.	%						
					(P)	(O)	(PP)	(D)	(OO)	(PPO)	(P_4)
19. VII. 09.	1	—	*Inoculated* with 10 c.c. of blood from the ear-vein of Dog 1.								
	2	103·0									
	3	102·6									
	4	103·0									
	5	104·0									
	6	103·8									
	7	102·8									
	8	103·0									
	9	103·0									
	10	106·0	*Parasites appeared:* few at film-edge.								
	11	106·0	... ...	0·5	2	27	64	1	2	1	3
	12	106·2	... ...	0·4*	·	68	26	·	2	2	2
	13	104·2	... ...	0·2	2	44	46	·	2	·	6
	14	103·0	... ...	2·4*	6	56	32	·	·	·	6
	15	102·8	... ...	0·8	10	41	42	2	2	·	3
	16	104·2	... ...	1·6	6	48	37	1	2	·	6
	17	105·0	Dog not feeding; looks ill (escaping pyriform parasites)	5·2	8	30	56	1	1	·	4
	18	102·0	... ...	20·0	6	41	44	1	2	·	6
6. VIII. 09.	19	—	*Dog found dead* at 10 a.m. (urine normal in bladder).								

* In this case 50 parasites were counted for types and the percentage of infected r.b.c. was estimated, 2,000–5,000 r.b.c. being counted by "fields."

Test Dog II. Spaniel, about ½ year old. *Inoculated* with blood taken from the ear-vein of Trypanblue-treated Dog 3 on the 190th day after the latter was inoculated.

Date	Day	Temp.°F.		% infected r.b.c.	%				
					(P)	(O)	(PP)	(D)	(P_4)
25. IX. 09.	1	—	*Inoculated* with 10 c.c. of blood from ear-vein of Dog 3.						
	2	—							
	3	102·4							
	4	101·6							
	5	101·6							
	6	102·8							
	7	103·2							
	8	104·4							
	9	103·4							
	10	104·6							
	11	104·4	*Parasites appeared* 4 (PP), 2 (O).						
	12	104·8	... ...	·05	·	·	·	·	·
	13	105·8	... ...	9·2*	2	46	49	2	1
	14	105·0	... ...	4·5	8	28	57	·	7
	15	104·6	... ...	5·0	9	41	46	1	3
10. X. 09.	16	—	*Dog found dead;* haemoglobinuria noted at autopsy.						

* Count only approximate, based on a poorly stained film.

Test Dog III. Fox-terrier, about ½ year old. Inoculated with blood taken from the ear-vein of Trypanblue-treated Dog 4 on the 182nd day after the latter was inoculated.

Date	Day	Temp. °F.	
25. IX. 09.	1	—	*Inoculated* with 10 c.c. of blood from ear-vein of Dog 4.
	2	—	
	3	101·4	
	4	101·8	
	5	103·0	No parasites found.
	6	102·8	,, ,,
	7	102·4	,, ,,
	8	104·2	,, ,,
	9	103·4	,, ,,
	10	102·8	*Parasites appeared:* few found.
	11	103·4	,, ·2 % r.b.c. infected.
	12	104·0	,, 2·4 % ,, ,, Blood thin.
7. X. 09.	13	98·4	,, 18·0 % ,, ,, Dog very weak, has haemoglobinuria. *Died* 8.45 p.m. of piroplasmosis. Many parasites found in smears from heart and spleen; few parasites in smears from liver and kidney.

Test Dog IV. Large mongrel, old. Inoculated with blood taken from the ear-vein of Trypanblue-treated Dog 6 on the 165th day after the latter was inoculated.

Date	Day	Temp. °F.		% infected r.b.c.	%				
					(P)	(O)	(PP)	(D)	(P_4)
25. IX. 09.	1	—	*Inoculated* with 10 c.c. of blood from ear-vein of Dog 6.						
	2	—							
	3	102·4							
	4	102·2							
	5	105·6	No parasites found.						
	6	104·0	,, ,,						
	7	103·4	,, ,,						
	8	104·6	,, ,,						
	9	104·2	,, ,,						
	10	104·0	,, ,,						
	11	104·2	,, ,,						
	12	104·4	*Parasites appeared:* few at film-edge, 1 (PP), 2 (O).						
	13	104·8	,, few at film-edge: 5 (PP), 2 (O).						
	14	105·6	... ...	·4		+	+		
	15	105·6	... ...	·5	1	56	39	1	3
	16	105·4	... ...	·3	2	48	47	3	·
	17	104·8	Few parasites at film-edge ...	+		+	+		
	18	104·4	... ...	·2		+	+		
	19	104·4	... ...	+		+	+		
	20	104·6	Haemoglobinuria	·7*		+	+		
15. X. 09.	21	—	*Dog found dead* of piroplasmosis.						

* Count based on a poor blood-film; the percentage probably too low.

Test Dog V. Terrier, about 1½ years old. Inoculated with blood taken from the ear-vein of Trypanblue-treated Dog 6 on the 210th day after the latter was inoculated.

Date	Day	Temp. °F.		% infected r.b.c.	%						
					(P)	(O)	(PP)	(D)	(OO)	(PPO)	(P_4)
9. xi. 09.	1	—	*Inoculated* with 10 c.c. of blood from ear-vein of Dog 6.								
	2	—									
	3	—									
	4	101·2									
	5	101·4									
	6	—									
	7	102·4	*Parasites appeared:* 3 (PP), 1 (O) at film-edge.								
	8	103·0	Parasites few: 10 (PP), 12 (O)	+		+	+				
	9	102·0	,, ,, 10 (PP), 8 (O) at film-edge	+		+	+				
	10	102·2	Parasites few: 8 (PP), 12 (O) at film-edge	+		+	+				
	11	102·2	... ...	·3*	2	80	12	·	4	·	2
	12	103·6	Dog lively from beginning	·2	·	77	20	·	2	1	·
	13	103·4	... ...	2·0	1	47	49	1	1	·	1
	14	104·8	Blood watery; dog lively, feeds well	1·2	5	60	29	3	·	·	3
	15	103·2	,, ,, ,, ,,	·2*	2	72	14	·	2	6	4
	16	101·8	,, ,, ,, ,,	·15	·	+	+	·	·	·	·
	17	103·0	,, ,, ,, ,,	·3*	·	62	36	·	2	·	·
	18	105·0	,, ,, ,, ,,	·1*	·	64	28	·	·	·	8
	19	104·0	Ditto. Very few parasites at film-edge	+	:	:	:	:	:	:	:
	20	104·4	Ditto	+	:	:	:	:	:	:	:
	21	103·0	,, ...	+	:	:	:	:	:	:	:
	22	102·8	,, 8 (O) at film-edge	+	:	+	:	:	:	:	:
	23	103·0	,, 10 (O) and 2 (PP)	+	:	+	+	:	:	:	:
	24	102·8	,, 9 (O) at film-edge, dog feeding ...	+	:	+	:	:	:	:	:
	25	103·4	Blood watery, dog feeding	1·0	3	53	29	1	7	·	7
	26	102·4	Blood watery, feeds poorly	·2*	·	82	13	·	1	·	4
	27	102·0	Ditto	·8	2	71	21	3†	1	·	2
	28	102·6	,, ...	1·3	1	56	24	1	2	·	16
	29	102·0	Blood very watery, feeds poorly	·7	·	67	18	2	10	·	3
	30	100·0	Ditto	·5*	·	70	24	2	4	·	·
	31	101·8	Ditto, very weak and ill, many nucleated r.b.c.	·3*	4	70	24	·	·	·	2
	32	101·4	Ditto	·7*	4	54	38	·	4	·	·
	33	95·8	,, ...	3·8*	2	62	20	2	10	·	4
12. xii. 09.	34	—	*Dog found dead.* At autopsy the blood was found very watery and the animal was profoundly anaemic. The spleen was slightly enlarged, the liver yellowish, the kidneys very pale and yellow. The bladder contained slightly haemoglobinuric urine.								

* 50 parasites counted for types, 100 counted in the other cases.

† One of these a (DD).

CONCLUSIONS REGARDING THE FOREGOING EXPERIMENTS.

It is evident from these experiments that the parasites persist in the blood of dogs which have recovered from an acute attack of piroplasmosis in consequence of treatment with Trypanblue and Trypanred. The blood of such dogs was found to be infective and virulent 143 to 210 days after the treated dogs were first inoculated. In respect to the persistence of the parasites in their blood, the successfully treated dogs behave similarly to dogs which have recovered naturally. Two of the dogs which recovered after treatment were reinoculated, with negative results, on the 213th and 221st day after the primary inoculation which produced the acute attack from which they recovered.

The enumerations of types of parasites in the case of Test Dog V are remarkable in so far as they show an exceptional preponderance of (O) parasites over (PP) forms, especially on days 11, 12, 15 and 26. In this respect the counts on these days approximate to those observed in trypanblue-treated dogs during the period when the (PP) parasites are disappearing. In Test Dog V the (PP) parasites, however, never fell below 12—13 %. In view of this appearance, coupled with the chronicity of the attack and the small percentage of infected r.b.c. recorded, it would appear as if some unfavourable condition for the multiplication of the parasites existed in this dog.

4. Confirmatory Experiments upon the Curative Treatment of Canine Piroplasmosis by means of Trypanblue.

Jowett (x. 1909), working on behalf of the Department of Agriculture, Cape Colony, has repeated and fully confirmed the experiments of Nuttall and Hadwen upon the cure of canine piroplasmosis in dogs by means of Trypanblue. The results of Jowett's experiments are here presented in the form of an abstract.

To avoid the occasional abscess formation at the seat of the dye injection, he recommends intravenous injection of Trypanblue whenever possible, this procedure having already been adopted by Nuttall and Hadwen in the treatment of cattle with the dye. Jowett's results may be summarized as follows:

I. RECORDS OF EXPERIMENTAL CASES.

Experiment A. Three dogs were each inoculated with 1 c.c. of virulent blood subcutaneously.

Dog 1. Fox-terrier. Weight 13 lbs. Fever on the afternoon of the 5th day.—Parasites appeared 7th day.—On the 8th day 0·75 °/₀ of r.b.c. infected.—On the 9th day 2·3 °/₀ of r.b.c. infected, dog very ill; *treatment:* 5 c.c. of saturated solution of trypanblue subcutaneously.—On the 10th day, no parasites found in blood-films, 14 hours having elapsed since treatment; dog very ill.—11th day, temperature 102·6°—102° F., dog lively.—No parasites were observed up to the 30th day the dog making an uninterrupted recovery. An abscess at seat of drug-injection healed speedily after being lanced.—On the 36th day the dog weighed 15 lbs. (=2 lbs. gained). *Result:* recovery satisfactory and complete. No loss of condition. Subsequently reinoculated with negative result.

Dog 2. Irish terrier (mongrel). Weight 36 lbs. Parasites appeared on the 8th day; fever.—On the 10th day 0·4 °/₀ of r.b.c. infected, dog obviously ill; *treatment:* 10 c.c. sat. sol. of trypanblue subcutaneously.—11th day no parasites found.—12th day, temperature 102·6—102·6° F., dog lively, no parasites.—13th day, painful swelling at site of dye-injection, lanced and dressed.—Dog made uninterrupted recovery, no parasites found on 14th to 20th, nor on 22nd to 23rd days.—Record ends 30th day. *Result:* "Recovery satisfactory and complete."

(Control) Dog 3. Fox-terrier (mongrel). Parasites appeared on the 8th day, 0·5 °/₀ of r.b.c. found infected.—9th day dog very ill, haemoglobinuria present; 7 °/₀ of r.b.c. infected.—10th day r.b.c. mostly infected, *dog died* of piroplasmosis.

Experiment B. Two puppies were each inoculated with 1 c.c. of virulent blood subcutaneously. (Blood of Dog 1, prior to treatment.)

Dog 4. Puppy, aged 2 months. Weight 3½ lbs. Parasites appeared on the 8th day.—On the 9th day 0·4 °/₀ of r.b.c. infected; *treatment:* 3 c.c. of 1 °/₀ sat. sol. trypanblue subcutaneously.—10th day no parasites.—11th day no parasites, puppy lively.—Recovery uninterrupted to 28th day, when record ends. No swelling at site of dye-injection. *Result:* speedy recovery.

(Control) Dog 5. Puppy, aged 2 months. Weight 4 lbs. Parasites appeared on the 8th day.—On the 10th day puppy very ill, 20 °/₀ of r.b.c. infected.—*Found dead* of piroplasmosis on the 11th day.

Experiment C. Two puppies were each inoculated with 2 c.c. of virulent blood subcutaneously.

Dog 6. Puppy, aged 2 months. Weight 3½ lbs. Parasites appeared 5th day, 0·5 °/₀ of r.b.c. found infected.—On 6th day 1 °/₀ of r.b.c. infected, dog obviously ill; *treatment:* 3 c.c. of 1 °/₀ sat. sol. of trypanblue subcutaneously.—On 7th day temperature 102—102° F., no parasites found, dog ill.—8th day, dog better, no parasites.—9th day, apparently recovered, no local swelling.—19th—20th day showed uninterrupted recovery without loss of condition. *Result:* Rapid and satisfactory recovery.

(Control) Dog 7. Puppy, aged 2 months. Weight 3½ lbs. Parasites appeared 6th day.—By the 9th day 0·5 °/₀ of r.b.c. infected; dog very ill.—10th day 3 to 4 infected r.b.c. per field.—12th day no parasites.—From now on to the 19th day temperature varied from 97° to 102° F., and few or no parasites found.—Puppy became emaciated, jaundiced and very anaemic. *Died* on night of 20th day.

Experiment D. Conducted on three dogs.

Dog 8. 6 months old. Weight 17½ lbs. Received 2 c.c. bloody serum from pup No. 5 at death. Intravenous inoculation. Parasites found on the 5th day, dog very dull.—6th day dog obviously ill, had haemoglobinuria, 9·5 °/₀ of r.b.c. infected; *treatment:* 4·5 c.c. sat. sol. of trypanblue subcutaneously.—7th day still very ill, no parasites.—8th day temperature 102·2—102° F., no parasites, dog better. 10th—18th day temperature normal. *Result:* "seems to have made an excellent recovery."

Dog 9. Puppy, 2 months old. Weight 4 lbs. Received 2 c.c. of bloody serum from pup No. 5 at death. Subcutaneous inoculation. *Treated on the third day after inoculation and before parasites had appeared. Result:* No parasites had appeared by the 16th day.

Dog 10. Terrier (mongrel). Weight 15¼ lbs. *Treated before inoculation.* The dog received 15 c.c. of 1 °/₀ sat. sol. of trypanblue injected subcutaneously. Six days later 1 c.c. of virulent blood (from Dog No. 8, before treatment) was inoculated intravenously. *Result:* The dog's temperature was irregular for some days, but parasites could not be detected in its blood. This dog is to be tested at a later date to determine if it has been rendered immune.

Experiment E. Conducted on three dogs.

Dog 11. Pointer puppy, 4 months old. Weight 18½ lbs. Received 1 c.c. of blood from Dog 8 before the latter was treated. Intravenous inoculation. On the 4th day the dog was obviously ill, 13·3 °/₀ of r.b.c. found infected; *treatment:* 1 fluid ounce of sat. sol. of *trypanblue per os.*—5th day, haemoglobinuria, r.b.c. mostly infected; no blue colouration of skin or mucous membranes. *Result:* 6th day dog found dead with typical appearances of piroplasmosis.

Dog 12. Pointer puppy. Weight 19½ lbs. Inoculated intravenously (saphenic vein) with 1 c.c. of blood from Dog 8 before the latter was treated. Parasites found on 4th day, puppy dull.—By the 6th day the dog looked very ill, temperature 105—105° F., more parasites present; *treatment:* 5 c.c. of trypanblue solution injected intravenously.—7th day temperature 101·4—102·4° F., dog lively and feeding; no parasites found. The record of this dog ends on the 9th day, the blood examination still negative. *Result:* dog apparently recovering.

(Control) Dog 13. Puppy, 2 months old. Weight 4 lbs. Inoculated subcutaneously with 1 c.c. of blood from Dog 8, before the latter was treated. Parasites found on the 7th day.—8th day 1·3 °/₀ and 9th day 9 °/₀ of r.b.c. found infected.—10th day dog dying, haemoglobinuria present, r.b.c. mostly infected. *Result:* dog died with typical appearances of piroplasmosis.

II. RECORDS OF CLINICAL CASES.

The following records refer only to dogs, six in number, which acquired the disease under natural conditions, i.e. by means of infected ticks. The dogs were handed over for treatment when very ill, two of them were moribund. "The remainder (four) were very well-marked cases, and we fully believe that had they not received the trypanblue treatment all these animals would have succumbed to the malady." In Jowett's experience, natural recovery "is very rare indeed," and he inclines to the belief that the disease arising from infection through ticks is more severe than that following upon inoculation with blood.

Dogs 1 and 2 treated when moribund. One died 2 hours after treatment, the other after 24 hours.

Dog 3. Fox-terrier. Weight 14½ lbs. Had been sick for some days, very ill on admission, temperature 104·4° F., haemoglobinuria present, parasites found. *Treatment:* 5 c.c. sat. sol. of trypanblue subcutaneously. 1st day after treatment, temperature 101—104·2° F., very ill, no parasites found. 2nd day, 102—102·8° F., much better, painful swelling at seat of drug-injection. 3rd day, 102—100·8° F., "quite lively and well," swelling lanced. From now on to the 28th day there were occasional rises of temperature. Infected r.b.c. were again noted on the 17th (0·7 $^0/_0$), 18th and 19th days.

Dog 4. Irish terrier. Weight 32 lbs. Obviously ill on arrival, 7 to 8 infected r.b.c. per film, temperature 103·6°, anaemia and haemoglobinuria present. *Treatment:* 10 c.c. of sat. sol. of trypanblue. 1st day after treatment very ill, weak and staggering, terribly anaemic, temperature 95—98·4°, no parasites found. *Treatment:* 2 c.c. of sat. sol. trypanblue after subcutaneous injection of 200 c.c. of normal NaCl solution. 2nd day, no parasites, dog better, temperature 101·2°. 4—6th day, dog improved, slough at seat of drug-injection; no parasites found.

Dog 5. Airedale terrier. Weight 35 lbs. Very ill on arrival, parasites found, haemoglobinuria present. *Treatment:* 13 c.c. of sat. sol. of trypanblue subcutaneously. "Parasites disappeared with a corresponding improvement in the dog's condition." Swelling at site of injection subsequently lanced. "The dog is now progressing satisfactorily."

Dog 6. Newfoundland. Weight 65 lbs. Very ill indeed on arrival, had been ill a week, parasites found in fair numbers; haemoglobinuria present. *Treatment:* 20 c.c. of sat. sol. of trypanblue intravenously. 1st day after treatment slightly better, only 1 (PP) infected r.b.c. found in film, blue colouration of dog only slight; treatment repeated: 5 c.c. of dye solution again injected. No swelling formed at site of dye-injection. "At the time of writing the animal is progressing satisfactorily towards recovery."

Summary of Jowett's results in the treatment of Canine Piroplasmosis with Trypanblue.

I. *Experimental cases.* Six dogs, inoculated with virulent blood and afterwards treated with trypanblue, all recovered. Four control dogs (untreated) all died. One dog which received trypanblue by the mouth also died. One dog treated 3 days after inoculation remained well and never showed parasites. One dog which received a dose of trypanblue and which was inoculated six days later, remained well.

II. *Clinical cases.* Two dogs in which treatment was attempted when moribund died. Four dogs, very ill and manifesting severe symptoms of piroplasmosis (anaemia, haemoglobinuria, icterus), all recovered after treatment with trypanblue.

I would note that the period of observation after treatment has been very short in a number of Jowett's cases and that some of the animals may have since had a relapse, such as we observed in two of our dogs (Nos. 10 and 11, pp. 184—186, this volume)[1].

5. **Summary and Conclusions.**

The following summary and conclusions apply to the results of the *experimental treatment* of canine piroplasmosis by various authors including myself:

1. Drugs which have been found ineffective in the treatment of canine piroplasmosis are:

Arsacetin tested on 1 dog by Nuttall and Hadwen (1909, p. 158).
Soamin tested on 1 dog by Nuttall and Hadwen (1909, p. 158).
Sodium-methylarsenate tested on 2 dogs by Nuttall and Graham-Smith (1908, p. 222).
Tartar emetic tested on 2 dogs by Nuttall and Graham-Smith (1908, p. 221).
Sodium nucleinate tested on 2 dogs by Levi della Vida (1907, p. 359).

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[1] In a short paper, which has just appeared, Jowett (25. XII. 1909) does not record any relapses. He states that the treatment has "more than realized our expectations." The paper referred to concerns itself with the dosage and the method of injecting the dye and was written with a view to informing veterinarians at the Cape regarding the treatment. The paper does not contain anything new. See my note on the solubility of the dye (*Parasitology*, II. p. 266): Jowett now uses 1—2 % solutions of Trypanblue (filtered in the latter case); the solutions are made up with boiling water.

Beta-naphthylamine tested on 2 dogs by Nuttall and Graham-Smith (1908, p. 223).

Brilliant Green tested on 2 dogs by Levi della Vida (1907, p. 359).

—— tested on 1 dog by Nuttall (1909, p. 413, this paper).

Benzopurpurine tested on 1 dog by Nuttall (1909, p. 414, this paper).

Tryparosan tested on 2 dogs by Nuttall and Hadwen (1909, p. 233).

Methylene blue tested on 2 dogs by Levi della Vida (1907, p. 359).

—— tested on 2 dogs by Nuttall and Graham-Smith (1908, p. 222).

2. Of doubtful value are:

Fowler's solution tested on 1 dog by Levi della Vida (1907, p. 360). The dog is stated to have had but a mild attack of piroplasmosis.

Atoxyl tested on 6 dogs by Gonder (1907) with negative results in that all of the dogs died of piroplasmosis. Levi della Vida (1907, p. 360) states that he cured some dogs by atoxyl treatment, but does not state how many.

Quinine bihydrochloride tested on 1 dog by Nuttall and Graham-Smith (1908, p. 221) with negative result. Memmo, Martoglio and Adani (1905, p. 23) state that they cured 2 dogs by injections of quinine, but give no particulars of their experiments.

Oil of Turpentine tested on 3 dogs by Levi della Vida (1907, p. 359) with negative results. Memmo, Martoglio and Adani (1905, p. 23), however, claim to have cured all of the 12 dogs they treated with turpentine.

3. Apparently the following drugs may exert some effect:

Dichlorobenzidine + 2 *molecules of amido-napthol-disulpho*, tested on 12 dogs, two of which recovered, Levi della Vida (1907, p. 360).

Sodium cacodylate tested on 7 dogs, 6 of which are stated to have recovered, Levi della Vida (1907, p. 360). (I am about to test this remedy afresh, since the Italian author gives very few particulars regarding his experiments.)

Congo Red tested on 3 dogs by Nuttall (p. 415, this paper). All of the dogs died, but the drugs exerted a decided effect upon the parasites.

4. Definite curative or preventive effects are exerted by:

Trypanblue ($C_{34}H_{24}N_6O_{14}S_4Na_4$, the tetrazo compound of toluidine and amidonaptholsulphonate of sodium) cured 7 out of 9 dogs in the experiments by Nuttall and Hadwen (VI. 1909, p. 175) and when given early (IX. 1909, p. 229) prevented the appearance of the parasites in the dogs' blood. These results have been confirmed by Jowett (X. 1909) who tested the dye on 14 dogs, two of which were moribund. The latter died whilst 12 recovered. Six of Jowett's cases were due to experimental infection, the others were cases of naturally acquired infection, and a number showed severe clinical symptoms. The drug is ineffective when given by the mouth. (Nuttall and Hadwen, IX. 1909, pp. 231—233; confirmed by Jowett, X. 1909.)

Trypanred tested on two dogs by Nuttall and Hadwen (VI. 1909) caused the parasites to degenerate and disappear. (Further experiments with this dye are in progress. The initial experiments indicated that trypanred is not as satisfactory as trypanblue for purposes of treatment as it produces a more irritant effect.)

5. In dogs which have recovered from piroplasmosis after treatment with Trypanblue and Trypanred the blood remains infective and virulent for 6 to 7 months or more after treatment. Such dogs resist reinoculation with virulent blood obtained from dogs suffering from acute piroplasmosis and, consequently, are to be regarded as "salted" animals. From a practical point of view the "salting" of dogs (and cattle) by the method of inoculation followed by the dye treatment appears to offer decided advantages. The method is at present on its trial in respect to cattle which are being "salted" in England prior to their exportation to Africa.

6. In judging of the value of dyes in the treatment of piroplasmosis it is essential that accurate observations should be made with regard to their *influence upon the parasites* as well as upon the symptoms of the disease. Both in dogs and cattle the injection of trypanblue causes the pyriform parasites to disappear rapidly, the remaining parasites appear

rounded and degenerated and ultimately disappear from microscopic observation. Although the parasites may reappear in small numbers after some days their presence is not accompanied by outward symptoms and the animals proceed to recovery. Relapses may, however, occasionally occur with the reappearance of parasites. Fatal relapses have only been observed in two of our dogs. The influence of the treatment upon the fever is very marked. Both in dogs and cattle suffering from piroplasmosis, the temperature usually falls rapidly to normal, and coincident with the fall in temperature there is a general improvement in the appearance of the animals. The effect of the dye appears to be to cut short the acute attack and to convert it into a mild, chronic form of the disease, which seems to exert no injurious influence upon the animals. In fact, the animals may rapidly improve in condition as evidenced by a gain in body-weight and in appearance. Having recovered from the acute attack the animals harbour the parasites in small numbers for a long time and by virtue of the continued presence in their bodies of the piroplasms they continue, for a like period of time, to remain resistant to reinfection. Whereas the animals cannot be described as "cured" in the sense that they are rendered free from parasites, they are rendered proof against reinfection; in other words they are "salted."

Judging from the immediate effect of the dye upon the parasites and upon the symptoms of piroplasmosis both in dogs and cattle and the subsequent behaviour of the animals after treatment, I am fully convinced that trypanblue will prove of great value in practice until such time as we shall discover an equally efficient remedy not possessing the colouring properties of the dye. The fact that trypanblue dyes the tissues for a considerable length of time will necessarily militate to some extent against its usage in cases where cattle are intended for meat-consumption, but it will assuredly protect valuable breeding animals which it is proposed to introduce into a country where piroplasmosis prevails. In the case of dogs, this objection will scarcely weigh in the balance as against saving the animal from a disease which is so commonly fatal. That *the colouration of the tissues disappears with time* is clear from the case of Dog 6 (see p. 422) which was killed on the 236th day after the dye was injected and found to be quite normal in appearance. Only further observations can determine how long the colouration of the tissues lasts in cattle and dogs and it would be well if different observers recorded their experiences in this particular.

At present there exists, to my knowledge, no other remedy which will cure piroplasmosis in either cattle or dogs.

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Blanchard, R. (v. 1909). *L'Insecte et l'Infection*, histoire naturelle et médicale des arthropodes pathogènes. Premier fascicule: Acariens. 160 pp., 197 illustrations in the text. Paris: Librairie Scientifique et Littéraire.

This fasciculus, the first of a series which will deal with arthropods in relation to disease, concerns itself with the Ixodoidea. The work promises to be very useful.

Braun, M. and Lühe, M. (1909). *Leitfaden zur Untersuchung der Tierischen Parasiten des Menschen und der Haustiere für Studierende, Ärzte und Tierärzte.* 186 pp., 100 text figures. 8vo., Würzburg—Curt Kabitzsch, (A. Stuber's Verlag).

Any work from the pens of Professor Max Braun and Dr M. Lühe is sure to be sound, careful, accurate, and helpful. The present work proves no exception to the rule. It contains a well thought out account of the chief Parasites of Man and domesticated and other animals. Careful instructions are given where to find and how to preserve and mount these parasites. These instructions are full and detailed, and cannot fail to be of great value to the doctor, medical officer of health, or the veterinary surgeon. Protozoan parasites have been dealt with by Dr Lühe, whilst the Metazoa are described by Professor Braun. The recent advances in our knowledge of Parasites carried by insects has necessitated an account of such biting Arthropods as ticks, mosquitoes, and other members of the Diptera and Hemiptera. The book is handsomely illustrated. We are happy to state that an English translation is fast advancing towards completion.

Guiart (1909). *Précis de Parasitologie*, 628 pp., 549 figures noires et coloriées. Boards: 12 fr. Paris: Librairie J.-B. Baillière et fils, 19 rue Hautefeuille.

The volume just issued by Professor Guiart is exceptionally welcome. It is well written, well printed, and well illustrated. It covers the whole ground of human parasitology; beginning with the parasitic fungi, passing on through the protozoan parasites, the account of which is as complete as our present knowledge admits, then through the various metazoan entozoa, and finally it even deals with the Arachnid and other Saprophytes of the cadaver. The book is in most respects abreast of recent knowledge, though the confusion in which the life-histories of Protozoan parasites is still environed renders it doubtful if in all cases Professor Guiart's account exactly corresponds with what really goes on in Nature. That, however, is not his fault. It is simply due to what Dr Johnson called our "sheer ignorance."

One of the features of the book which especially attracts us is the novelty of its illustrations: many of them are new, many of them are taken from recently published scientific papers, and the well worn "hack" figure is mercifully spared us.

We have in fact nothing but praise for the book, with the solitary exception that we should have liked to have had one of the figures taken from our own work attributed to its source.

PROWAZEK, S. VON (1909). *Taschenbuch der mikroskopischen Technik der Protistenuntersuchung.* 2nd ed. Leipzig: Verlag von Johann Ambrosius Barth. 87 pp. 17 × 11 cm. Price 2.50 Marks. Cloth.

The second edition of this useful little pocket-book will be welcomed by protozoologists since it contains many additions to the previous edition. To our knowledge there is no other book of the kind. We can safely recommend it to workers in the laboratory.

THIMM, C. A. (1909). *Bibliography of Trypanosomiasis.* Issued under the direction of the Honorary Managing Committee of the Sleeping Sickness Bureau, London. 228 pp. 25 × 16 cm. London: Sleeping Sickness Bureau, Royal Society, Burlington House, W. Price (Paper cover) 4*s.* net.

This bibliography, compiled by the Librarian of the Bureau, will prove quite invaluable to those interested in trypanosomiasis since it contains nearly 2000 titles of publications arranged both according to authors and to the journals in which they appeared. All papers which appeared before April 1909 are included and references to Glossina literature are given. The costs of publications have been heavy and the price of the bibliography is not calculated to do more than cover a part of the costs of production.

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By GEORGE H. F. NUTTALL, M.D., PH.D., Sc.D., F.R.S., CECIL WARBURTON, M.A., F.Z.S., W. F. COOPER, B.A., F.Z.S., F.L.S., and L. E. ROBINSON, A.R.C.Sc. (London)

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